

nature

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How companies benefit from the CERN connection

EVERYONE knows that the non-stick saucepan was a technological spin-off from the Apollo space programme, and everyone knows that you have to think quite hard if pressed to name a second product with which the ungrateful world of consumers has been accidentally endowed by high technology. But space exploration is not without its benefits of a sort. The vast technology-based industries that have sprung up to serve space and for that matter, military needs, can be seen as (if nothing else) a means of keeping large numbers of people in employment. Inevitably, however, one asks whether the development, say, of better space propulsion units by industry, when there is only one customer, serves any broader purpose, and almost equally inevitably one answers that it does not. The rapid break-up of many space-and-defence-based industries when the big client loses interest or runs out of money is testimony to the problems of industry capitalising on the push of technical demand when that push is on too narrow a front.

Not every major new project based on science and technology comes from aerospace, however. It is fascinating to ask whether anything rubs off on an industrial company which performs contract work in the construction of nuclear reactors, radiotelescopes, deep-sea drilling equipment or whatever: and a fascinating answer is given in a recent and highly readable report published by CERN (*A Study of Economic Utility resulting from CERN contracts*, H. Schmied, Report no. CERN 75-5). The diligent Dr Schmied conducted 110 interviews with CERN staff and ultimately identified 127 companies throughout Europe where there was a possibility that dealings with CERN had created 'utility' for the company beyond the contract itself. The utility could spring either from increases in added value in subsequent sales of products elsewhere or from cost savings in subsequent operations. Increases in added value might arise from the sales of new products originally developed for CERN, increased sales of existing products owing to the CERN connection, improvement of a product to meet a tough specification and subsequent sales of the improved product, or by other means. Cost savings could arise from the indirect use of CERN expertise in research, development and production, from savings on capital investment when CERN contracts had already partially covered costs and from savings in marketing from the use of CERN as a reference.

Companies were asked to make their estimates (using guidelines provided) of the utility of having worked on contract. Obviously, and Dr Schmied acknowledges as

much, there is no foolproof way of identifying utility; some companies find it difficult to accept that anyone beyond their own four walls could possibly have contributed anything of significance, others might think a high value for utility would please CERN and perhaps land more contracts whilst yet others might think a high estimate could cause CERN to adopt a more hawkish attitude in its future letting of contracts. And the measurement of utility is itself bound to be a fairly hit-and-miss affair. Nonetheless there are some very striking conclusions.

If all 127 companies are taken together, their sales to CERN amounted to 394 million Swiss francs (MSF), but the utility generated came to 1,665 MSF: the CERN connection had borne fruit fourfold. There are obvious gradations. Manufacturers of standard electrical equipment such as cables have little to gain from working for CERN. At the other extreme manufacturers of computers and precision engineering reported an average of 17 and 32 respectively for the utility/sales ratio. And companies in the UK, Norway, Sweden and Denmark have done particularly well, undoubtedly because the CERN contract has opened up new geographical market horizons.

This study does not say that the world is economically a better place because of CERN, or that economic activity is created which would not have existed otherwise; it is entirely conceivable that a study of companies unfavoured by such contracts could identify losses to counteract the gains of the favoured companies. What it does say is that individual companies demonstrably profit in many ways from association with large-scale high technology. And this is particularly interesting in CERN's case, because none of the companies seems to have been exclusively dependent on CERN for its turnover, and a very large part of the utility achieved has necessarily to be outside the narrow realms of particle accelerators.

It would be wrong to take these figures as a good measure of the extent of technology transfer possible through major technological enterprises, as some of the utility has been gained by the simple process of stamping "as supplied to CERN" on the glossy brochures. But industrialists could well take note of the financial advantages of working with high technology. And governments involved in big projects should ponder whether the acquisition of large numbers of their own technical staff for specific jobs is the best way to take the broadest possible national advantage of the investment.



Net profits

C. E. Purdom and A. Preston of the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Lowestoft, write about the prospects for fish farming in the UK.

THE annual world production of farmed fish is approximately 4×10^6 tons which represents about 15% of supplies of fish for direct human consumption. In the UK, production at present is about 1,500 tons a year, or less than 0.2% of consumption. Precise statistics are not available, but a considerable proportion, perhaps half, of the production is for conservation or sport purposes.

The fish farming industry in the UK comprises a variety of small, mostly privately owned, farms and a few large companies. About 100 private farms exist, each producing between 5 and 200 tons per year with by far the greater proportion at the low end of the scale; in addition, about 30 farms under the control of Regional Water Authorities produce fish for re-stocking purposes. Small scale fish farming has a history going back about 100 years. It is traditionally associated with the production of trout for sport purposes, although current expansion also includes food production. Involvement by large companies is very recent and is still in an early developmental phase, with production primarily for food supplies. Output is still low and geared to the supply of luxury food in the form of trout and salmon, with research emphasis on the latter. So far there is little input into the mass retail industry and marketing methods remain traditional.

Apart from a very minor effort with cyprinid fish for re-stocking, fish farming in the UK is at present restricted to salmonids. The culture in fresh water of native brown trout (*Salmo trutta*) and North American rainbow trout (*S. gairdnerii*) is the dominant element, with sea-water culture of salmon (*S. salar*) restricted to large companies with production levels of only a few hundred tons per year at present.

Marine fish farming research in the UK was initiated by the Ministry of Agriculture, Fisheries and Food (MAFF) about 15 years ago but its origins go back much further than this and probably to the turn of the century when hatcheries were established for plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) to produce fry for liberation into the sea for conservation purposes. This ineffective measure was discontinued by about 1920, but the concept of farming marine fish seems

to have grown out of the experience gained in the production and hatching of eggs of the plaice. Thus initial research was hatchery orientated and directed towards plaice farming. Hatchery techniques were successfully developed and large scale production of juvenile plaice became feasible by 1966. At this time the White Fish Authority (WFA) entered the field to develop the rearing techniques towards commercially viable levels and, especially, to study the growing-on phase from juvenile stages to marketable size. It was in this area that economic problems with plaice became apparent; their food bill exceeded the first-sale value. Attention was therefore switched to sole (*Solea solea*) and turbot (*Scophthalmus maximus*) for which better economic returns might arise. Sole has proved more intractable than plaice during on-growing but turbot has been shown to be very amenable during this part of its life cycle. A basic requirement in intensive fish farming is that food is taken rapidly and not wasted or allowed to decompose and pollute the environment. Turbot fulfil this requirement but sole, with present rearing techniques, do not, being slow, cautious feeders. Progress in turbot farming has been restricted, however, by serious difficulties encountered in the production of juvenile fish and present research on farming techniques depends, in part, on the collection of juveniles from wild stocks. For conservation reasons this sets a restrictive limit on possible total commercial production for this species of about 100 tonnes a year. The earliest attempt to rear turbot was made by Anthony in 1910 but the first hatchery reared juveniles were not produced until 1972 and then only in very small numbers. Improvements have been made in succeeding years, during which the development of turbot hatchery techniques has had considerable priority in government laboratories, but the methods still fall short of the level of achievement necessary for commercial exploitation, although one major food company is exploring the hatchery production of turbot with the assistance of MAFF.

Fish farming in the UK has grown slowly in the freshwater and marine salmonid field under commercial patronage, although some acceleration has occurred over the past 2 or 3 years; on the other hand, government research, largely under the auspices of MAFF, but with the later involvement of the WFA and the Natural Environmental Research Council (NERC), has been preoccupied with the development of methods for rearing marine flatfish. So far no major research work on salmonid farming has been undertaken by government agencies, and commercial interest in marine flatfish has been

limited, largely to a joint project between the WFA and one major food company. This dichotomy is in marked contrast to the situation in other parts of the world, such as North America, Japan and Norway, where government and private concerns are mutually and extensively involved in a wide variety of fish farming projects.

With little prospect of increasing the direct yield from the sea of species of prime fish, and many indications that the reverse might occur, it has become necessary to examine the potential for an expansion in fish farming in the UK. In this context a review was commissioned with the object of drawing up a MAFF programme of research and development into marine and freshwater fish cultivation and assessing the facilities required to carry it out. This review, which took into account the existing scope of fish farming activities, formed the view that a viable industry needed to be broadly based and to cater for both the small farmer and major commercial interests. Some legislative adjustments may be necessary in this context but the primary objective should be to provide, through a well conceived research and development programme, a sound scientific basis for the expansion of fish farming into a broadly based national industry.

The question of which species of fish to cultivate is crucial to the development of a research and development programme of relevance to an expanding industry. Among the salmonids, rainbow trout and Atlantic salmon are obvious choices in view of their present commercial status; brown trout and sea trout may also be of value within the context of marine culture of salmonids. Among the marine flatfish, turbot alone seems to have potential at this time, although sole would have definite economic advantages if the feeding problems experienced in the growing-on phase could be solved. Other freshwater and marine species do not warrant attention at present but should be subject to periodic review in terms of their potential for cultivation.

The research and development needs differ between species. The principal subjects areas for investigation are hatchery studies, nutritional requirements, water management studies, facility development, genetic manipulation and disease control. Of these, hatchery techniques, nutrition and disease control are well established for salmonids after more than a century of development. There is still room for much improvement, particularly at the nutritional level, and disease control and treatment require continuous study. The major constraint to a large scale expansion in trout farming in fresh water, however, lies in the nature of the water requirements. Rainbow

trout farms use about 30,000 gallons of high quality fresh water for each pound of fish produced and the availability of sites for expansion is very limited. Two methods for overcoming this constraint are the use of recycling systems and the culture of trout in sea water. The former can be regarded as a technological development based on present practices, whereas sea-water culture is a more radical change with many facets requiring investigation. Although no major research work on salmonid farming has been undertaken by MAFF or the Department of Agriculture and Fisheries for Scotland some facilities are available and plans for additional facilities are in hand: in these, special attention will be directed towards nutritional studies, cage systems in the sea, recirculation systems for fresh and salt water, and genetic studies aimed at producing improved strains. Disease research and monitoring is already covered both in England and Wales and in Scotland.

Flatfish farming research has not yet established a sufficiently reliable technology for full commercial development. The first priority for further

work lies with turbot and especially with the need to overcome the problems of hatchery production of juveniles.

One major area of work of fundamental importance to any form of fish farming is the development of a genetic approach to produce improved strains, to avoid inbreeding depression and to conserve genetic uniformity and hence performance in domesticated lines of fish. A further useful field with genetic implications is the development of all-year-round spawning stocks and the control of sex itself. Genetic manipulation at the hatchery level is at present studied within the MAFF programme, but no facilities exist for extending this to the growing-on phase or for holding stocks of domesticated fish. For marine flatfish, the creation of domesticated lines has not yet begun. Similarly, Atlantic salmon stocks are basically of wild origin but rainbow trout exist in a wide variety of selected lines, particularly in North America. Although a proper evaluation of their merits, and the possibility of heterosis in F1 hybrids, seems not to have been attempted, this is an area of study

which could have an immediate beneficial effect on farming practices in the UK.

In conclusion, there is an established and expanding industry for salmonid farming, principally in fresh water, and a growing interest on the part of large companies in marine flatfish culture. In view of the likely diminution of supplies of prime quality fish like sole and turbot from the sea it is desirable to encourage the continued expansion and development of this area of food production, on a broad basis and at all levels of commercial involvement, though this should not be construed as implying any substantial contribution to offset a short-fall in basic supplies. To this end the programmes of research and development in the fields of hatchery production, environmental control, nutritional innovation, genetic manipulation and disease identification and control within the species at present exploited or showing potential for exploitation require careful integration. The list of species at present includes rainbow trout, salmon and turbot with secondary consideration for brown trout, sea trout and sole. □

THE term "vitamin(e)" was coined by Casimir Funk in 1912. He was good at promoting its use, but he could never have foreseen the mind-expanding effect of this new word in the 1970s. Vitamins are coenzymes and other metabolic catalysts whose pathways of biochemical synthesis have been lost as a result of mutations during the evolution of animals. Identification and synthesis of vitamins led to spectacular successes in preventive medicine; these became well known, and, as one result, popping vitamin pills is part of the American way of life, just like colour television in the family room. The idea is not so much that diets are deficient, but why not take vitamins "just to be safe"? Indeed, except for a half-hearted attempt some years ago by the US Food and Drug Administration (FDA) to throw cold water on it, the habit had few opponents and it was certainly better than smoking cigarettes.

There is, however, a basic pharmacological delusion of the layman that if a small dose of a medicine is good, a large dose must be even better, especially if it is not poisonous. Many physicians have treated patients whose symptoms exceeded in severity their physical diagnosis, by prescribing "a good rest with lots of vitamins". This procedure is known as "placebo therapy"; the administration of a harmless nostrum for soothing the nerves of the apprehensive, and for convincing those who are open to

Funk therapy



THOMAS H. JUKES

suggestion that they are receiving "medical treatment". Vitamins can be sold without prescription, and the medical profession, by fostering the use of vitamin pills, made an opening for nonphysicians to practice medicine without a licence. The "health-food" industry responded, and its sales outlets stock pills containing vitamins and alleged vitamins. If health food is so "nutritious", why does it need supplementation with large doses of vitamins and minerals? The answer given in the health food literature is that such dosage will produce super-health, an effect not measurable in human beings or laboratory animals.

Vitamins go through periods of

fashion. Thiamine was in the spotlight at one time and, more recently, ascorbic acid became the darling of the headlines. Vitamin B₁₂ is popular, probably because it is often injected and is red. Vitamin B₆ is all the rage now, and vitamin E is a hardy perennial because it was lucky enough to be discovered by the fact that its deficiency causes infertility in rats. The same is true of vitamin A, of course, but E has ever since worn the halo of Aphrodite, and has collected many unearned credits.

"Megavitamin therapy" has its devotees, and publicity is currently given to "orthomolecular psychiatry", defined as the "achievement and preservation of good mental health by the provision of the optimum molecular environment for the mind, especially the optimum concentrations of substances normally present in the human body, such as the vitamins". Whether or not this actually works we shall probably never know, because the author (Linus Pauling) also says "the principles of medical ethics prevent orthomolecular psychiatrists from withholding from half of their patients a treatment that they consider to be valuable. Controlled tests can be carried out only by skeptics".

Efforts of the FDA to regulate megavitamin promotion, however, were set back by a court decision and by the passage last year of a bill in the US Senate that specifically prevents the FDA from classifying high-potency vitamin preparations as drugs.

international news

THE Review Conference of the Commonwealth Agricultural Bureaux, which has just ended in London, could be a landmark in the history of the organisation. The CAB was set up in 1929 to provide an administrative centre for the group of bureaux, each of which acted as a clearing house for information in a different branch of agricultural research. Since then the group has expanded, and it now comprises nine bureaux, which function as originally intended, and four institutes, which have somewhat different functions. Although only an 'organisation', in the loosest sense, the bureaux have maintained, and justified, their reputation for providing the world's best and most comprehensive sources of information in the fields they cover. Nonetheless, and perhaps because of their very success, the bureaux have in recent years appeared to be somewhat conservative, even at times complacent, as regards their own functions and performance.

The CAB has three rather distinctive functions:

- A global documentation service covering virtually the whole field of agricultural and allied research in a series of abstracting journals.
- A taxonomic and identification service, provided by the three institutes dealing respectively with entomology, mycology and helminthology.
- An operational service provided by the Commonwealth Institute for Biological Control.

From these springs a fourth function, the operation of a 'mutual assistance' agency that provides Commonwealth countries with very much more in the way of information and services than they could obtain for themselves at anything like the same cost and effort.

The annual budget of the CAB totals slightly more than £3 million, of which about half comes from the subscriptions of member countries, and another £1.5 million from the sale of bureaux publications. Only about 30% of the output goes to Commonwealth countries, and because these can buy their information at a reduced rate, no less than 85% of the income from these services comes from outside the Commonwealth. The major 'foreign' clients are the United States, Japan, and one or two European countries such as the Netherlands and Germany. The question of the cost-effectiveness

CAB move with the times

from a correspondent

of the bureaux operations was a principal subject of discussion.

Taking first the documentation services, it was felt that these could and should be more self-supporting than at present, particularly in view of the increasing cost of production and materials. So the CAB has been taking a hard look at some other internationally used abstracting services, such as International Retrievals (IRS), who are believed to make a profit, and INSPEC, which claims to break even, more or less. One result was a resolution that the bureaux should "seek to associate themselves with non-Commonwealth partners, national and international, as consortia". This is already done with Food Science and Technology Abstracts, providing an international food industry information service in consortium with the German company IDW.

One obvious development ought to be collaboration with the AGRIS system now being sponsored by the FAO from Rome. The CAB sees itself as complementary to, certainly not competing with, AGRIS, but the strong anti-national prejudice that at present surrounds all UN-oriented operations would probably make close collaboration impossible at present.

On the technical side, the CAB intends to make much more use of modern computerised production methods, including increased use of machine-readable material, which the bureaux have been noticeably slow to adopt.

The situation as regards the work of the three institutes is very different from that of the bureaux. Their services have always been given free, only the Commonwealth Mycological Institute having a small income from the sale of cultures of fungi. A small step is now being taken, whereby a charge of £10 an item will be levied for all identifications done for non-contributing governments or com-

mercial clients. The case of the Commonwealth Institute for Biological Control (CIBC) is different again. A suggestion that its headquarters might be moved from Trinidad back to the UK was resisted by the representatives of the less developed Commonwealth countries. But there was general agreement that the institute should in future concentrate on fewer, bigger projects rather than providing a 'fire service' to deal *ad hoc* with any problem that might arise within its terms of reference.

In line with efforts to streamline the services of the CAB, the CIBC's outstations will also be reduced to four in number, strategically situated in accordance with a new, more closely regional policy. They will be sited in Trinidad, in Delemont, Switzerland (for forest pests mainly in North America) in Kumasi, Ghana and in Bangalore. Smaller outstations will be run down and, in future, work not done at the four main centres will be on an *ad hoc* project basis.

Finally, there are to be changes and expansion in the central management of the CAB. The present small secretariat, with only four senior officers, will be reinforced by the appointment of a marketing manager (although in deference to the inhibitions of some of the older bureaux staff, his official brief will be "circulation and services") two editorial managers and a scientific coordinator. More problematic will be the question of regrading and even redundancy in certain staff categories. This may well have to wait until some of the more conservative among the present Directors of Bureaux have retired. The fact is that these have hitherto traditionally been senior scientists with great experience in the discipline for which their bureaux are responsible. But under modern conditions, what is probably needed is a group of highly expert information scientists, ready to take advantage of every new aid to quicker, more efficient production of the Abstracts, more prepared to enter into agreements with other organisations and perhaps less inclined to feel that if the CAB doesn't do it, it isn't worth doing. Many of the changes that must come will have to be made anyway, and in most instances the sooner the bureaux can meet changing circumstances half way, the better for everyone. □

Dr Gerald Stanhill has recently come to the conclusion that his Biblical and post-Biblical ancestors showed remarkable powers of scientific observation and analysis. Dr Stanhill, who heads the Division of Agricultural Meteorology in Israel's Agricultural Research Organisation, points out that the traditional Jewish prayers for rain, codified almost two thousand years ago, are most frequent and most intensive at precisely those times of year when additional moisture will do most to raise wheat yields in the Holy Land.

Scientific studies carried out over the past 20 years indicate that above average rainfall in the early winter immediately after sowing increases yields, as does bountiful precipitation after the wheat has flowered. In contrast, rainfall at the beginning of the season (before sowing has taken place) and in midwinter (between tillering and booting) depresses yields. And, indeed, prayers for rain become most frequent and high pitched just after sowing (which took place in Biblical times, and takes place now, in November). If rain does not fall in spite of the prayers, a series of fasts is prescribed: at first only for the sages, then for the entire public from dawn to dusk and finally, if the dry spell continues, 24 hour fasts are to take place twice each week.

Both religious practices and archaeological digs indicate that Jewish farmers of the Biblical period were growing wheat primarily in the southern part of the country (where rainfall is both scarce and uncertain) or in the hilly regions (where the soil is thin and does not absorb a great deal of moisture at one time). Today's most prosperous agricultural areas, where rain can be counted upon, were not cultivated for the most part by the Israelites. Interior lowlands, like the Huleh and Jezre'el Valleys, were soggy swamps, and remained so until they were drained a few decades ago.

● While the draining of the swamps increased the supply of fertile farmland and contributed to the elimination of malaria, it has not always proved an unmixed blessing. As a result of the elimination of the Huleh Swamp, for example, the Sea of Galilee is facing a pollution threat.

The peat of the former swampland is heavily impregnated with nitrogen, and when water flows through the Huleh and into the Sea of Galilee, some 15 km to the south, it carries with it large quantities of nitrogen. This influx, many scientists believe, increases the growth of aquatic vegetation in the water, thus contributing to the pollution of Israel's central fresh water reservoir.

A number of researchers have suggested that the problem be solved by planting crops in the Huleh Valley which would consume the excess nitrogen. Dr Avraham Hartzook of the Agricultural Research Organisation, for example, has in mind a plant known as Kenaf (*Hibiscus cannabinus*), which would not only deal with the nitrogen question, but might also reduce the country's dependence

Letter from Israel

from Nechemia Meyers



on increasingly expensive and scarce wood pulp for the production of paper.

Some time ago Hartzook helped to introduce Kenaf to Tanzania, where he spent two years as an Israeli agricultural expert. Now a successful cash crop in that country, its fibres primarily serve as a substitute for jute, but they have also been used, albeit on a small scale, as a substitute for wood pulp by the paper industry. Dr Hartzook sees a considerable potential for Kenaf in Israel, which has little wood of its own and might therefore be ready to exploit the plant earlier than other, more richly endowed countries.

● Pollution problems in the Gulf of Eilat are, if anything, more serious than those in the Sea of Galilee according to Professor Ralph Mitchell, now on a working visit to the Weizmann Institute from Harvard University.

Mitchell, who has done extensive studies of the Gulf under the sponsorship of the United States Office of Naval Research, says that if pollution, particularly oil seepage, continues unchecked, the beautiful coral reefs of the area will be eaten away in 25 years.

Mitchell has also done studies on how oil affects the biological behaviour of inter-tidal snails. Some stop bunching together (to keep from being washed away), and others simply stop eating.

Oil likewise affects the ability of bacteria to detect their food, thus inter-

fering with their vital role as marine self-purifying agents. Following the discovery that motile marine bacteria are chemotactic to many of their food sources, Mitchell demonstrated that sub-lethal concentrations of oil inhibit this chemotactic process so that the rate of substrate decay is retarded.

Mitchell is concerned not only by oil seepage, but also by the possibility of a major oil spill from one of the big tankers that wend their way to Eilat.

● Israel's mathematics teachers have established their own emergency group to fight a Ministry of Education ruling that mathematics will no longer be a compulsory matriculation subject for secondary school students.

Cutting the teaching of mathematics, they argue with great vehemence, would undermine secondary schools and hamstring universities, forcing the latter to disregard matriculation certificates and devise a battery of entrance examinations. Even worse, according to the teachers, the Army would find itself short of men with the kind of mathematical background required to handle increasingly sophisticated modern weapons.

The controversy can only be understood against the background of Israel's educational system, which differs considerably, for example, from that in the UK. Students are not given an 11-plus examination, and when they finish elementary school, a great majority continue on to a secondary modern school.

The last two years of secondary school do bring a measure of specialisation similar to that at A-level in Britain. But, even so, students concentrating on mathematics and science have thus far had to pass courses in history and literature, while those interested in the humanities have had to prove their abilities in science and mathematics in order to obtain a matriculation certificate.

Though there had long been complaints about the burden these requirements imposed on students, demands for a change only came to a head several years ago when the Education Ministry revealed that 40% of the humanities students were failing matriculation mathematics.

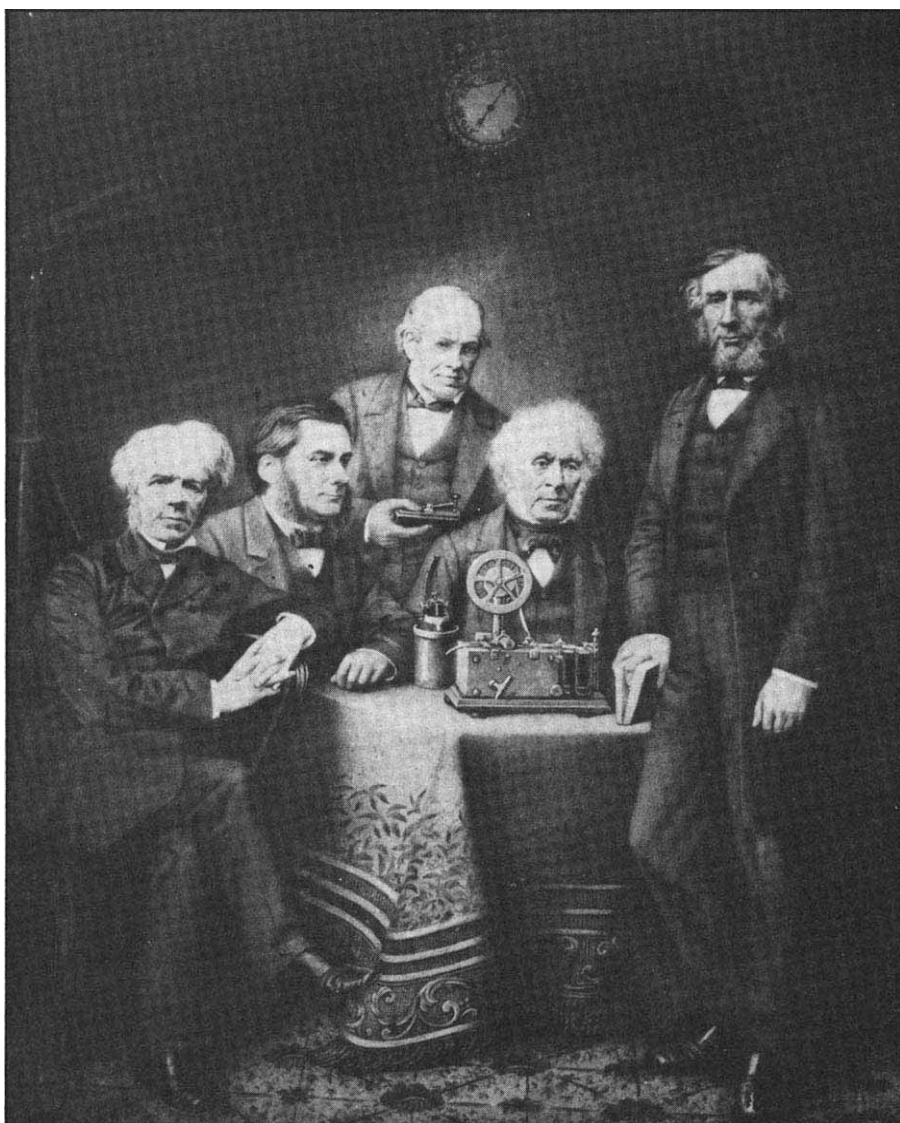
As an interim measure, the examination was made easier, thus allowing a much larger percentage of examinees to slip under the wire, but this only disguised the problem without solving it. So the ministry decided on more drastic action: students in the last two years of secondary school, it announced, could choose to study maths at various levels (the higher the level, the more points towards a matriculation certificate), or not study maths at all.

Wheatstone's wheezes on show

by John Hall

It comes hard to be told that Sir Charles Wheatstone actually didn't invent the bridge of the same name, but such was the range of that scientist's inventiveness and imagination that one feels he must, on balance, be forgiven what amounts to a fairly minor omission. Bridges apart, he made fundamental contributions to the study of electrical measurement in the 1830s and 1840s, and in the process he happened across principles of transmitting electrical impulses which formed the basis of modern telecommunications systems. As if that were not enough, he spent his twenties experimenting with musical instruments, and managed as the *pièce de résistance* of this period an invention which was to become the fisherman's friend. Letters patent granted by George IV in 1829 described his creation as "a certain improvement or improvements in the construction of wind instruments"; it was the concertina, successor to earlier brainchildren like the 32-key Wheatstone symphonium (pocket-sized and powered by breath), and precursor of mighty wheezers like the 81-key Wheatstone duet, with the range of a piano. The inventor's nail fiddle, a poor relative of the African thumb piano, made of nails to be scraped with a violin bow, was less of a raging success.

But Wheatstone of Wheatstone's Music Shop, Islington, clearly had a novel turn of mind, as the scientific community discovered when he was appointed Professor of Physics at King's College, London. His interest in the transmission of sound vibrations led him to experiment with the transmission of electrical impulses, and to measure their speeds along wires. His success in sending information by this means led to the needle telegraph on which the developing railway system came to depend for its signalling, the ABC telegraph, an automatic Morse transmitter, a punched-tape Morse transmitter and Wheatstone's printing telegraph, which in operation was akin to a Telex printer. For the record, Wheatstone's contribution to the measurement of voltage, current and resistance was that he produced an accurate calibrated variable resistance to replace the unreliable galvanometric instruments available at the time. Later prophets will also be sad to learn that the linear motor was invented by Wheatstone in 1842; given a larger current, it might have proved his brightest wheeze.



He was also responsible for the introduction of the Royal Institution's practice of locking lecturers in a room immediately before they are due to lecture; apparently, on one occasion when he was about to give a public discourse, Wheatstone's nerve went at the last minute, and he disappeared from the scene, leaving Faraday to *ad lib* the evening away.

Wheatstone is one of two eminent Victorian members of King's College to be honoured by an exhibition there this summer. The other is Sir Charles Lyell, an equally prominent and worthy man in his field of geology, but affording rather less tangible tokens of his life's work than does Wheatstone. Lyell established the main principles of geology, and amazed the learned of his day by showing that the Earth was about 4,000 million years old when you would have sworn it wasn't half that age. This scale managed to accommodate the Darwinian theory of evolution, and also gave cosmological theories a realistic time perspective, but doesn't sit conveniently in an ex-

hibition display cabinet. By way of compensation there are some nice photographs of the Lyell family seat, a moderately accomplished watercolour by the man himself, and a body of evidence which testifies to the meticulous nature which the documentation of his theories must have demanded. Even as a child he collected shells and bones for his private museum, and when he wrote home to Papa it was in Latin. But he was not such a scholar that he closed his eyes to the exigencies of the world outside his books; after a page and a half of Latin, written from Midhurst Grammar School in 1814, he blurts out in the mother tongue: "If I write any more in Latin I shall miss the post."

The exhibition, at King's College, Strand, London, continues until September 10.

The picture shows the shining lights of Victorian science (left to right): Michael Faraday, Professor T. H. Huxley, Sir Charles Wheatstone, Sir David Brewster and Professor John Tyndall. □

THE British government is to draw up codes of practice for some experiments involving the genetic manipulation of microorganisms which could pose a potential hazard to workers and the general public. Mr Fred Mulley, Secretary of State for Education and Science, in answer to a parliamentary question, announced the setting up of a working party which would

- draft a central code of practice and make recommendations for the establishment of a central advisory service for laboratories using the techniques available for such genetic manipulation, and for the provision of necessary training facilities.

- consider the practical aspects of applying in appropriate cases the controls advocated by the Working Party on the Laboratory Use of Dangerous Pathogens, chaired by Lord Godber, which reported earlier this year.

Mr Mulley also asked the Research Councils and others concerned not to proceed with work already identified as involving a potentially serious hazard, pending advice from the work-

ing party.

The question of potential hazards inherent in the new techniques which allow DNA of different bacteria and viruses to be linked together and introduced into other bacteria was con-

Government codes for gene manipulation

by Eleanor Lawrence

sidered by the Working Party on the Experimental Manipulation of the Genetic Composition of Microorganisms, set up by the Advisory Board for the Research Councils and chaired by Lord Ashby. This committee, which reported in January of this year, came to the conclusion that such experiments should be permitted but only with certain safeguards.

These conditions include the use of experimental microorganisms which have been rendered relatively innocuous by specific mutations, and that certain

types of experiment should be carried out under conditions of the strictest bacteriological security, in laboratories where the risk of escape to the outside or contamination of workers is minimised.

The definitive code of practice approved by the government, which the new working party should provide, will be welcome since it should end a period of delay and uncertainty which started a year ago with a call for a moratorium on a range of experiments by a committee of the National Academy of Sciences in the United States. At the same time the working party will be concerned with the practical aspects of implementing the findings of the Godber Committee which considered the use of proven pathogenic bacteria and viruses in laboratories throughout the UK. This committee drew up a list of organisms which could only be worked within conditions of strict security, which in the UK would confine such work to the few existing laboratories with suitable facilities. □

Shutting the GATE

by John Gribbin

THE World Meteorological Organisation (WMO) has been stirred into issuing a formal denial that the GARP Atlantic Tropical Experiment (GATE), conducted last year by ten nations and probably the largest and most complex international scientific experiment ever undertaken, was an experiment in hurricane control which adversely affected weather conditions in some countries.

The need for this denial stems, according to the WMO, from a statement made by "a professor of the

University of Mexico", and the WMO says that "it would indeed be regrettable if such unjustified statements were to detract from the acknowledged success of this international scientific research venture". Since the purpose of GATE was to monitor natural changes in the atmosphere and oceans, including large scale tropical disturbances, it would have been rather stupid of the investigators to attempt to alter the natural state of affairs, and most meteorologists are well aware of the need to understand natural changes in atmospheric processes before trying to induce changes by artificial means.

Perhaps the professor was confused by the inclusion of the word "experiment" in GATE's name which, al-

though in the acronymous tradition of the WMO, is perhaps a slightly misleading title for an entirely observational project. But since Mexico was one of the participating countries, it should not have been too difficult for the critic to check his facts before issuing a public statement. The lesson to be drawn, perhaps, is that research into weather and climate is such a sensitive area that bodies such as the WMO should expect to be subject to the same intensive scrutiny as agencies responsible for nuclear research, or laboratories involved in genetic engineering.

In that case, perhaps GARP Atlantic Tropical Study might have been a happier choice of name. □

correspondence

PAC report: a failure of communication

THE Indian Public Accounts Committee report "Foreign Participation or Collaboration in Research Projects in India" (discussed in a leading article on July 31) was critical of the Bombay Natural History Society, as well of the Research Unit for Genetic Control of Mosquitoes. In this letter, Dr A. N. D. Nanavati, Secretary of the Bombay Natural History Society, considers some implications of the report.

THE Public Accounts Committee (PAC) has expressed grave doubts about a number of research programmes carried out with foreign collaboration in India. Much of their alarm stems from the fact that the knowledge obtained from such researches can have applications in chemical and biological warfare (CBW) and that the administrators examined by them did not seem to be fully aware of the fact. Based on this finding the committee has formed an opinion implied, though not explicitly stated, that those concerned in these

projects were dupes, sometimes maybe even willing tools (in view of the relatively high salaries they received) in the hands of foreign organisations who wished to use India as an experimental arena for such studies.

I do not propose to go into the details or to try to refute the numerous misunderstandings and errors in the evidence on which these opinions were based. The facts of which I have personal knowledge, namely those connected with the Bombay Natural History Society (BNHS)—Migratory

Animal Pathological Survey (MAPS) Bird Migration Study, have already been stated in your columns (November 29 1974) and, I believe, adequately answer every criticism voiced against the study originally by Dr Jayaraman, and later endorsed by the committee.

I need not repeat these arguments here except to point out that Dr Salim Ali's *cri de coeur* to Dr T. Ramachandra Rao, then the Director of the Virus Research Centre (VRC), Poona, requesting assistance for the studies on ectoparasites and blood smears of ringed birds has been completely misinterpreted. At the outset of the programme between the WHO and the BNHS, studies on this aspect were made by the VRC, Poona, and details of their findings were published from year to year in the Annual Reports of the VRC. When the WHO withdrew from the study, the VRC also withdrew, as it had no staff to spare for this work. Apart from occasional seasonal trips by the Institute of Natural Foci of Infectious Disease (NFID), Omsk for collection of such material (reports published in Russian), no other organisation came forward and this material remained unstudied until the MAPS organisation came on the scene. After the first year, they also requested that no more blood smears be sent as they were not in a position to study them. It was this lack of interest, and not any secret removal of data, which occasioned Dr Salim Ali's appeal to Dr Rao (who also found himself unable to help). These "orphan" blood smears are still with the society, awaiting a godfather to take an interest in them. There are several other misconceptions in the report, for example, the importance attached to Dr Theiler's suggested hypothesis on Yellow Fever epidemiology. This remains no more than a hypothesis, because it fails to explain all the observed phenomena. But these misconceptions are not of primary importance. What is important is that the PAC did not examine any scientist directly working on the projects, except Dr T. Ramachandra Rao, the officer on special duty, whose position was rather peculiar and is further discussed later. Had they examined the scientists, rather than the administrators and administrative scientists, who were only nominally concerned with the actual work on the projects, they might have revealed a completely different picture. In the case of the BNHS study, an examination of the facts will readily show that every working scientist was aware of the possible implications and repercussions, that the programme of work was formulated by the Indian Chief Investigator, and that adequate safeguards against secrecy were in force.

This brings us to the question of how

far the administrator should be aware of the different facets and possibilities of any research study he is required to sanction. He must obviously rely on the opinions of his experts. Is it then incumbent on the experts concerned to appraise the administrator of all the various possibilities inherent in a proposed research study, and is such a procedure really necessary or relevant or even possible? When a project is being prepared with a particular object in mind, it is necessary to establish how far that the object will be met by the study. The expert has to advise on (a) the importance of the problem (so that it may be judged in proper perspective in the national context), (b) the type of information which needs to be obtained to solve the problem and (c) the extent to which the proposed study can be expected to yield the required information. This is all the administrator needs to know to enable him to assess the project correctly and to judge its requirement in men and materials and its anticipated benefits relative to the many other demands which may be made on his resources. Anything else is irrelevant. Considerations like possible misuse of the knowledge gained, the safeguards necessary, and so on, must be the responsibility of the experts on the job. For example, a few years ago a study was made on the spread of cholera from village to village in West Bengal and the mechanisms by which the village wells got contaminated. Every student of medicine and microbiology knows that the understanding of such process can be used either for countering the spread of cholera or for its enhancement, that is for biological warfare using cholera germs. Yet, had the administrators concerned been questioned about that project they would not have shown any greater awareness of such dangers than in the present case.

The committee's conclusions lose much of their significance because they were not, in fact, based on the evidence necessary for a correct appreciation, the evidence of the scientists concerned on the job. The only working scientist examined was Dr T. Ramachandra Rao, who made it clear that knowledge is neutral and the only safeguard against misuse of knowledge is its free publication.

The committee's criticisms and apprehensions about the detailed programme and sequence of studies made by the Research Unit for Genetic Control of Mosquitoes may or may not be correct. It is impossible to say without questioning the scientists concerned, which has not been done. Dr Rao could not say much about the formulation of the programme because he came on the scene after this work had been done. (It may be noted in pass-

ing that the remarks on the financial benefits supposed to have accrued to Dr Rao by joining the scheme are contradicted by the Indian Council of Medical Research (ICMR) letter, laying down the terms of Dr Rao's appointment.)

In spite of such errors and misconceptions, we must take due note of the fact that an independent body of men, representing all shades of opinion, has enquired into these projects and has found reasons for apprehension. Although some of these apprehensions may be exaggerated or based on incorrect evidence, there are others, not so prominently emphasised by the committee, which call for attention.

If we accept the proposition that the details of work, the implications of the study, and the safeguards against possible dangers are the responsibility of the expert concerned, it is also incumbent on the authorities to appoint proper and competent experts. Was this done? The committee has rightly pointed out that neither of the two individuals who occupied the office of the Director General of the ICMR (Indian counterpart of the Project Director) was qualified to judge the intricacies of entomological research. This was a serious lapse and though it was soon remedied, by the appointment of an eminent entomologist, Dr T. R. Rao, as an officer on special duty, such an appointment should have been made before any programmes were decided on. This delay may, as the Press Trust of India correspondent claimed, have involved us in programmes of dubious value. Whether this was or was not the case could not be ascertained from the evidence available. But that such a possibility existed is indicative of our failure to maintain adequate vigilance; this is the real danger which needs to be highlighted.

Unfortunately, the defect is a part of a larger malaise, and calls for examination of the defects in our methods of recruitment and appointment which leave a substantial proportion of our most brilliant men and women unemployed, so that they must seek opportunities for work in other countries. And what of our habit of vesting all authority in a top official irrespective of qualifications? But consideration of these points would not be strictly relevant to the issues raised in this report, however important they may be in creating such potentially dangerous situations.

It is unfortunate that although the committee has made sincere efforts to examine the issues involved, its conclusions are invalid because of a lack of appreciation of the distinction between the role of the administrator and that of the scientist in research projects. □

news and views

Hoyle on troubled waters

from John Faulkner

SIR Fred Hoyle (Cockley Moor, Caltech, Cornell . . .), the angry young man of British astronomy, turned a surprising 60 recently, and some seventy-five astronomers and astrophysicists met to celebrate the event on July 15–17 at a conference in his honour, "The Frontiers of Astronomy in 1975".

Isola San Giorgio, Venice was the apparently curious but in fact inspired choice of site for a meeting in which past achievements and the at times pessimistic future were surveyed. An attempt to ensure coverage of most areas in which Hoyle had contributed significantly meant that an unusually wide variety of topics were discussed.

In the popular mind, Hoyle is mainly known for his part in cosmological and other arguments. To the professional astrophysicist, however, his most important and enduring achievement must surely be his seminal contributions, going back three decades, to the theory of nucleosynthesis—the production of the elements—in both stellar and cosmological environments. It is worth stressing this point because Hoyle's ideas and their consequences have become such an accepted part of the "obvious" background for present studies that his role has to some extent suffered that curious extinction and obscurity which occurs when "everybody knows that". I have found that not a few professional astronomers are unaware of the 1946 (yes!) paper on the equilibrium process in supernovae and the abundances of the iron peak nuclei. Perhaps they can be forgiven—even Margaret Burbidge (University of California, San Diego) confessed that its importance only hit her six years later. Again, in the early fifties, Hoyle pondered the difficulty of producing the observed abundances of carbon and oxygen in reasonable stellar environments and was led, following Salpeter's identification of the normally unstable ^8Be nucleus as an intermediary, to propose the existence of a hitherto unsuspected energy level and its properties in the ^{12}C nucleus. Brilliantly confirmed by Whaling in Caltech's Kellogg Laboratory (despite the earlier scepticism of

his colleagues), this prediction had two important consequences: astronomy had been shown (again) to be a laboratory at large which could lead and stimulate, rather than merely follow, terrestrial physics; and it convinced Fowler (Caltech) to become a nuclear astrophysicist and ultimately led to the massive 1957 work of B²FH. Some young astrophysicists are unaware of the way in which their subject changed from an arid preserve of applied mathematicians into a dynamic part of modern physics. They have Fred Hoyle to thank in large measure for that change.

Fowler discussed recent experimental and theoretical work at the Kellogg Laboratory on the properties of light to medium nuclei. He emphasised that there were many low-lying excited states likely to be important in supernova events, but essentially inaccessible in the laboratory. Work such as Woosley's on the systematics of the nuclei had been very important in this regard, and calculated and experimental charged particle reaction rates were good to factors of ~ 2 up to ~ 3 billion degrees. With uncharacteristic pessimism however, he concluded that this global approach to understanding was probably at an end. His colleague C. A. Barnes added to the gloom by remarking that the solar neutrino problem is still with us. The hoped-for escape route by way of a resonance state in ^8Be (preventing the ^8B neutrinos from being produced) seems to have been killed by no less than three groups working with different reactions (led by Dwarakanath and Kavenagh at Caltech and Parker at Yale). On a brighter note Barnes mentioned that work by Rolfs (University of Toronto) and his Caltech colleagues, measuring reduced widths of states near particle thresholds by direct capture studies, had opened the way for determining cross sections for a whole host of astrophysically important reactions. Gloom returned when he remarked that the trend in cross sections for important carbon and oxygen reactions ran counter to other heavy-ion reactions and were something of a puzzle.

A shaft of Texan sunlight illuminated

the scene when Clayton (Rice University) with customary evangelical enthusiasm presented evidence and arguments that refractory grains formed in supernova or nova ejecta may have survived heating in the early solar nebula (*Nature*, in the press). Concomitantly (*Astrophys. J.*, **199**, 765; 1975), extinct ^{129}I may have decayed in the grains rather than in meteorites, as currently supposed. This could require a major revision of the early evolution of the solar nebula.

In another interesting paper Woosley (Caltech) discussed the nucleosynthesis that can occur in portions of the Universe characterised by relatively high baryon densities (a possibility recently put forward by Hoyle). In contrast with earlier calculations, Woosley was able to produce large amounts of elements heavier than iron, up to $A \sim 100$; indeed, the amount in the range $60 \lesssim A \lesssim 80$ could be embarrassingly greater than in the Solar System. With an intermediate baryon density (as in the Rees "chaotic" models), abundances in this range are quite consistent with those observed in very metal deficient Population II stars, as discussed, among other abundance data, by Pagel (Royal Greenwich Observatory).

On the (possibly) cosmological front, Hazard (Cambridge University) pointed out that the apparent strong degree of density evolution exhibited by QSOs could lead to strong fluctuations in their volume density. An identification programme of Molonglo sources carried out with Murdoch (Sydney University) indeed shows large variations of OSO surface densities for a given redshift in different regions. While it was too early to fall in with the conference spirit and drew sweeping conclusions from grossly inadequate data, the recent discovery (on the same night!) of the two new $z \geq 3$ objects at Lick and Steward Observatories encouraged optimism that significant studies might some day prove possible. Ekers (Groningen) recalled Hoyle's 1959 suggestion of a minimum in the angular size-flux density relation in certain cosmological models. Several hundred weak Westerbork sources (down by ~ 100

on the weakest 3C sources) show such an effect, at last. But the results are not in quantitative agreement with the expectations of simple, non-evolving cosmologies.

In other areas, Wickramasinghe (Cardiff) recalled early work with Hoyle on the nature of interstellar grains. In addition to grains including graphite, a suggestion of a component consisting of silicates covered by crystalline organic polymers is supported by recent data on the melting temperatures of cometary dust. Steigman (Yale University), discussing the interstellar medium as studied through the Copernicus satellite observations, was critical of their earlier interpretation. With Strittmatter and Williams, he has shown that circumstellar HII regions can account for almost all the absorption features observed; hence the bulk of the observed material may be circumstellar rather than interstellar. Solomon (SUNY, Stony Brook) from studying CO emission at $\lambda \sim 2.66$ mm (resulting from CO-H₂ collisions) has concluded that most of the interstellar medium in the interior of the galaxy is molecular H₂.

Stellar structure, in which Hoyle also played a large role, was rather under-represented at the meeting. Demarque (Yale University) gave a largely histori-

cal review. Faulkner (University of California, Santa Cruz) discussed Hoyle's stimulus on his own horizontal branch work, and recent developments in connection with the possible effects of gravitational radiation on close binary evolution. Arnett (University of Illinois) ruefully showed how six months work since the Dallas meeting (*Nature*, **253**, 231; 1975) had resulted in the calculations of supernova collapse and neutron star formation being pushed about a millisecond further.

Perhaps the most appreciated tribute to Hoyle was that in Rees' (Cambridge University) stimulating talk in which he announced that the main building at the Cambridge Institute of Astronomy would henceforth be known as the Hoyle building. "Being stimulating", said Rees, "can be more important than being right". When Hoyle concluded the meeting with a discussion of his most recent cosmological ideas (after expressing a hope that NASA and the NSF can survive the assaults of democracy) it was clear that many in the audience retained the right to be sceptical. But that did not stop them from rising to applaud him for his achievements (as aptly put by the host, Professor Rostagni at the start of the meeting) during the first sixty years of his time on Earth.

electric vector is parallel to the field line, it can shake an electron it meets up and down the magnetic 'wire' and can, therefore, be scattered by it. If, however, its electric vector is perpendicular to the field, it cannot shake an electron as it passes and will emerge without being scattered. Such an anisotropic scattering process clearly produces polarisation of the emergent X rays, even if initially the X rays had no net polarisation. Observation that the X rays from the pulsed X-ray sources are strongly polarised would lend strong support to the accreting magnetised neutron star models. In addition, observation of how such polarisation varies through the pulse cycle would give valuable information about the emission process itself.

A certain amount (though probably less than about 10%) of polarisation is to be expected in the X-ray flux from the non-pulsing sources as well. Material captured from the companion star is likely to have so much angular momentum that it cannot accrete directly on to the compact object. Instead it forms an accretion disk around it. Matter in the disk slowly spirals inward towards the central compact object, releasing energy in the form of X rays as it does so. If the compact object is a black hole, all the observed X rays come from the disk, but if it is a neutron star, about half of them come from a hot thin boundary layer where the disk grazes the stellar surface. In general, in the central region of the disks where the bulk of the X rays is produced, the disk is optically thick and the dominant opacity is due to scattering of the X rays by electrons. Suppose that we view such a disk from an angle, so that its apparent shape is an ellipse, and consider an X-ray photon emerging from the disk towards us. Before it was scattered out of the disk, the most probable direction in which it was travelling is upwards in the disk, that is, in a direction perpendicular to the plane of the disk. Since the electron which gave rise to this last scattering must have been shaken by the photon's electric field in a direction perpendicular to the photon's pre- and post-scattering paths, we see that the photon's electric vector must have been in the plane of the disk and perpendicular to our line of sight. Thus photons leaving the disk are preferentially linearly polarised, and the position angle of the polarisation gives us information about the orientation of the disk in space.

In such close binary systems, a fraction (a few per cent) of the X rays emitted by the compact object strike the companion star. Of these, a large proportion is scattered off the stellar atmosphere, while the rest are absorbed and converted into optical and

X-Ray polarimetry in astronomy

from J. E. Pringle

MOST of the known X-ray stars in our Galaxy are believed to be members of close binary systems, in which material from an ordinary star is being accreted on to a compact, X-ray emitting companion. Our present evidence on the nature of these systems comes from studies of the variability and spectral shape of the X-ray continuum and from observations in other parts of the electromagnetic spectrum, in particular at optical and infrared wavelengths. In recent articles, Alan Lightman and Stuart Shapiro (*Astrophys. J.*, **198**, L73-L75; 1975) and Martin Rees (*Mon. Not. R. astr. Soc.*, **171**, 457-465; 1975) have stressed the importance of making observations of the polarisation of the X-radiation from these sources.

The two X-ray stars which display regular pulses, Hercules X-1 and Centaurus X-3, are thought to be rotating, magnetised neutron stars. The material being accreted on to them is funnelled by their strong (about 10^{12} Gauss) magnetic fields and falls on to the star at the magnetic poles. There the energy of infall is released as X rays, and so

the poles act as two X ray emitting hot spots on the neutron star's surface. If the magnetic axis is not aligned with the star's rotation axis, then, as the star rotates, first one pole becomes visible and then the other, making the X-ray emission appear pulsed. Although the details of the X-ray emission process are not well understood, it is fairly certain that X-radiation produced in such a strong magnetic field should be strongly polarised. The cyclotron frequency for the electrons is in the X-ray range and the X-ray photons themselves are probably produced by emission at the cyclotron frequency or low harmonics thereof. Such cyclotron emission is itself highly polarised. The X-ray photons then have to find their way out from the emission region at the stellar surface through the column of accreting material which is falling down the magnetic field lines. In a strong magnetic field, the infalling electrons are essentially tied to the field lines like beads on a wire. Consider an X-ray photon travelling perpendicular to the field lines. If its

ultraviolet radiation. Such scattered photons are again likely to be highly linearly polarised. By arguments similar to those above it is clear that the net electric vector of the scattered photons would tend to be aligned with the rotation axis of the binary system. The amount of such polarised scattered X rays received would vary depending on how much of that part of the star that is illuminated by the X-ray source is visible—that is, it would vary with the orbital period. In addition, the smaller the angle between our line of sight and the rotation axis of the binary system, the greater the amount of time for which the illuminated part of the star is visible and hence the smaller the size of the variation. Thus, in principle, such an observation could tell us the inclination of the binary orbit to our line of sight. Because such a small fraction of the emitted X rays actually strikes the companion star, such an effect would, however, be difficult to detect.

X-ray polarimetry is at present in its infancy, but it seems likely to grow to maturity over the next few years. In the past, the best-known argument for X-ray polarimetry has been that it is often symptomatic of non-thermal synchrotron-type emission. Drs Lightman, Shapiro and Rees have drawn attention to other sources of X-ray polarisation and, more importantly, have shown how X-ray polarimetry can be used as a testing ground for theoretical models, and as a means of probing the structure, of the binary X-ray stars.

Ozone over Britain

from Peter D. Moore

It is no longer possible for the British to be complacent about ozone pollution. Though Los Angeles is still the worst hit area of the world, having occasional peaks of 70 p.p.h.m. of ozone in its air (Marx, *Science*, **187**, 731; 1975) and having at least once reached 99 p.p.h.m., the problem is far more widespread: even in Melbourne peaks of 28 p.p.h.m. have recently been recorded.

In the United States, ozone is regarded as by far the most damaging of air pollutants to plants. In New Jersey, Pell and Brennan (*Environ. Pollut.*, **8**, 23; 1975) consider that \$128,000 worth of damage was caused to agricultural and ornamental plants in 1972, and estimated that losses of over \$35,000,000 have been made for California. Ozone is not yet considered an economic threat to British agriculture.

In 1972, Atkins *et al.* (*Nature*, **235**,

372; 1972) demonstrated the production of atmospheric ozone over southern England and later Derwent and Stewart (*Nature*, **241**, 342; 1973) published data for central London in which they found daily peaks of 8–12 p.p.h.m. Recently Cox *et al.* (*Nature*, **255**, 118; 1975) have concluded that a proportion of southern England's ozone may have been transported considerable distances, of the order of 100–1,000 km. It seems clear, therefore, that Britain has an ozone pollution problem, though it is not entirely of her own making.

Bell and Cox (*Environ. Pollut.*, **8**, 163; 1975) have now published data, obtained in 1972, concerning plant damage by ozone in Britain. They exposed the ozone-sensitive variety of Bel-W3 of tobacco to the atmosphere in Ascot (32 km west of central London) and at the same time monitored the air for ozone. They observed the characteristic symptoms of ozone toxicity (necrotic spots on the leaf surface) and their atmospheric measurements showed that ozone levels had risen above 4 p.p.h.m. on 322 occasions between July 4 and September 15 and above 6 p.p.h.m. on 83 occasions. On one occasion a level of 10 p.p.h.m. was maintained for 5 h. (The United States air quality standard of the Environmental Protection Agency is 8 p.p.h.m. for one hour.) Bell and Cox confirmed that there was a strong positive correlation between the degree of leaf damage and the length of time for which each test plant had been exposed to ozone levels in excess of 4 p.p.h.m. More resistant tobacco varieties showed very low levels of damage and no significant correlation with ozone exposure; no correlation at all was demonstrable with sulphur dioxide concentration.

These results show that diurnal peaks in ozone, at levels which could be injurious to both man and plants, are not restricted to central London, but could be occurring over a much wider radius. No records have yet been reported of ozone damage to crops or to wild plants in Britain but, as Bell and Cox point out, growth may be suppressed without symptoms being visible.

Recent experiments on a number of herbaceous plants in America, however, by Harward and Treshow (*Environ. Cons.*, **2**, 17; 1975) suggest that visible symptoms and growth reduction are rare at levels of 5–7 p.p.h.m. ozone for about 2 h per day, but become apparent in some species when levels of 15 p.p.h.m. are used. In August 1973, levels as high as 17 p.p.h.m. were recorded at Harwell, 80 km west of London, so perhaps visible damage will soon make itself apparent and we can recognise that we do indeed have an ozone problem.

Agglutination of tumour cells

from Robert Shields

MUCH research effort has gone into investigations of why plant lectins often agglutinate tumour cells to a greater extent than their normal counterparts. As with all generalisations about tumour cells, enhanced agglutination is not an invariant property of transformation. It is, however, sufficiently widespread in the model systems used by cell biologists to have promoted intensive study.

Using the lectin concanavalin A (Con A) it has been shown that agglutinability of some lines of cells is directly related to their tumorigenicity when injected into animals (Inbar *et al.*, *Nature new Biol.*, **236**, 3; 1972). Also, cells transformed with temperature-sensitive tumour viruses exhibit high agglutinability at the permissive temperature (where the transformed phenotype is expressed) and lowered agglutinability at the non-permissive temperature (Burger and Martin, *Nature new Biol.*, **237**, 9; 1972; Eckhart *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 283; 1971). The experiments show that high agglutinability is closely associated with oncogenic transformation. This idea was considerably strengthened by experiments that used a variety of selective pressures to produce revertants of tumour cells with approximately normal behaviour. The revertants are less agglutinable and less tumorigenic than their transformed parents (Pollack, *In Vitro*, **6**, 58; 1970; Pollack *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **60**, 126; 1968). One of the best pieces of evidence for the close linkage of agglutinability and transformation is from work carried out by two separate groups (Culp and Black, *J. Virol.*, **9**, 611; 1972; Ozanne and Sambrook, *Lepetit Colloq.*, **2**, 248; 1971) on revertants. They used the fact that agglutination of tumour cells with high doses of Con A leads to cell death. After treating transformed cells with high concentrations of the lectin and isolating surviving cells, they were able to establish cell lines with reduced agglutinability and a more normal phenotype.

It has recently been suggested that tumour cells are highly agglutinable because they have more microvilli on their surfaces and that the presence of these microvilli is regulated by cyclic AMP (Willingham and Pastan, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1263; 1975). These workers suggest that microvilli help agglutination by bringing larger areas of cells' surfaces into close apposition, allowing them to be cross-linked by lectins. They suggest that it is the low level of cyclic AMP

in tumour cells which allows formation of microvilli and hence agglutination.

Using dark field microscopy these workers found that the transformed cells examined had many hairlike microvilli on their surfaces, and normal cells (in this case 3T3 cells) had none. But when 3T3 cells rounded up in mitosis or were treated briefly with trypsin numerous microvilli could be seen. These are precisely the conditions where cyclic AMP levels are low and the cells agglutinate readily. The concept that cyclic AMP could regulate agglutinability through microvillus formation was tested directly by incubating transformed cells for extended periods with dibutyl cyclic AMP, then examining their surface for microvilli and measuring agglutinability. After 24 h treatment the cells had few microvilli and were far less agglutinable. But the decreased agglutination of tumour cells after prolonged incubation with dibutyl cyclic AMP may have more to do with expansion of the G2 phase of the cell cycle (Smets, *Nature new Biol.*, **239**, 123; 1972) where transformed cells are less agglutinable (Smets and deLey, *J. Cell Physiol.*, **84**, 343; 1974) than any genuine reversion to a more normal phenotype. Also dibutyl cyclic AMP has been reported to affect the synthesis of some cell surface components (Goggins *et al.*, *J. biol. Chem.*, **247**, 5759; 1972), and it is possible that it interferes with agglutination through its effect on these molecules rather than by any direct action on microvilli.

Better evidence for the direct role of cyclic AMP in agglutination comes from the use of prostaglandin E_1 or the phosphodiesterase inhibitor isobutyl methyl xanthine. Brief treatment of transformed cells (L929) with either compound leads to a rapid increase in cyclic AMP as well as a decrease in microvilli and a decrease in agglutination. When the cells are washed free of these compounds cyclic AMP levels drop and microvilli and agglutinability return.

The idea that transformed cells are agglutinable because they have numerous microvilli is only the most recent of a long series of explanations, most of which have been oversimplified or erroneous. However, the theory does have the merit of being easy to test. It should be pointed out that not all tumour cells are more agglutinable than normal cells and not all cells that agglutinate have numerous microvilli. There is no reason to suppose that a single difference between cells is the reason for the difference in their agglutinability. It seems far more likely that agglutination is a very complex process involving factors such as microvilli, surface charge and density and mobility of lectin receptors and

only in a few model systems will one factor predominate. Perhaps only when agglutination is understood in simple cells such as the erythrocyte will factors that control agglutination in more complex cells be understood.

Around the world ballooning for dust

from David W. Hughes

BALLOONS have been used by scientists ever since the pioneering work of Etienne and Joseph Montgolfier in 1783, either to investigate the atmosphere or to lift apparatus above most of it. Modern plastic balloons provide an inexpensive vehicle that can lift thousands of kilograms above 99% of the atmosphere (to heights of ~ 33 km) or hundreds of kilograms above 99.9% (to ~ 50 km) and can carry the weight there for days.

One of the latest experiments, named Magellan, is jointly organised by Wlochowicz of the National Research Council of Canada, Ottawa and Hemenway, Hallgren and Tacketh of the Dudley Observatory, Albany, New York. Preliminary results were given at the IAU Colloquium no. 31 on Interplanetary Dust and Zodiacal Light recently held at Heidelberg. Magellan is designed to fly at an altitude of about 30 km and to collect relatively large solid particles, diameter 50–300 μm (mass 10^{-7} – 5×10^{-5} g), as they fall through the atmosphere.

As the flux of particles is low the experiment must have a large collecting area exposed for a long time, but the particles must be concentrated in a much smaller area for easy counting, photography and chemical analysis. To do this the Magellan balloon payload has a vertical conical mylar funnel (like an umbrella) the wide end being uppermost and 50 m² in area. The funnel apex has an angle of 60° and particles falling into the funnel slide down the sides into a sampling pan at the bottom. The payload has three sampling pans which can be exposed for pre-set periods of time and then sealed. The funnel is hung from a 300 m nylon line below the balloon to minimise contamination. The payload weighs 385 kg and contains an electronics package for tracking and for activating the cut-down mechanism.

In two engineering flights from Australia using a 20 m diameter super-pressure balloon one payload went all the way round the world and was cut down and eventually recovered within 15 km of the launch site. The second payload was not so well behaved, circling the Southern Hemisphere twice and staying up for 210 days before

finally falling into the Pacific ocean. Two short-duration flights from the NCAR Balloon Flight Station at Palestine, Texas, showed that about nine particles per hour were collected by the system. These particles are now being analysed to give incident flux, mass distribution and composition.

As in all new techniques there are problems. First, the particles can have different origins. They could be micrometeoroids (particles which do not reach boiling point on atmospheric entry and are retarded and fall slowly to Earth); they could be fragments of much larger meteoroids which disintegrated on entering the atmosphere; or they could be terrestrial contaminants which have fallen off the balloon or been blown up to 30 km from lower down. Only the micrometeoroid flux is known as this can be inferred from spacecraft data. The second problem is political. It is hoped that the work will not be hindered by nations reticent about balloons flying across their territory and that multinational cooperation will soon allow Northern Hemisphere flights to start.

Two-scale mantle convection

from Peter J. Smith

ALTHOUGH convection currents in the Earth's mantle have been the subject of extensive theoretical study and speculation, experimental investigations of convective models have been surprisingly few. Yet as Richter and Parsons (*J. geophys. Res.*, **80**, 2529; 1975) point out, laboratory studies have a crucial part to play in testing the stability of analytical and numerical models, especially of three-dimensional systems. In spite of previous work suggesting that the conditions for stable convection may be quite restrictive, the question of stability is all too frequently ignored—a situation which Richter and Parsons claim, somewhat wryly, “may in part be responsible for the proliferation of mantle convection models that bear little resemblance to one another”.

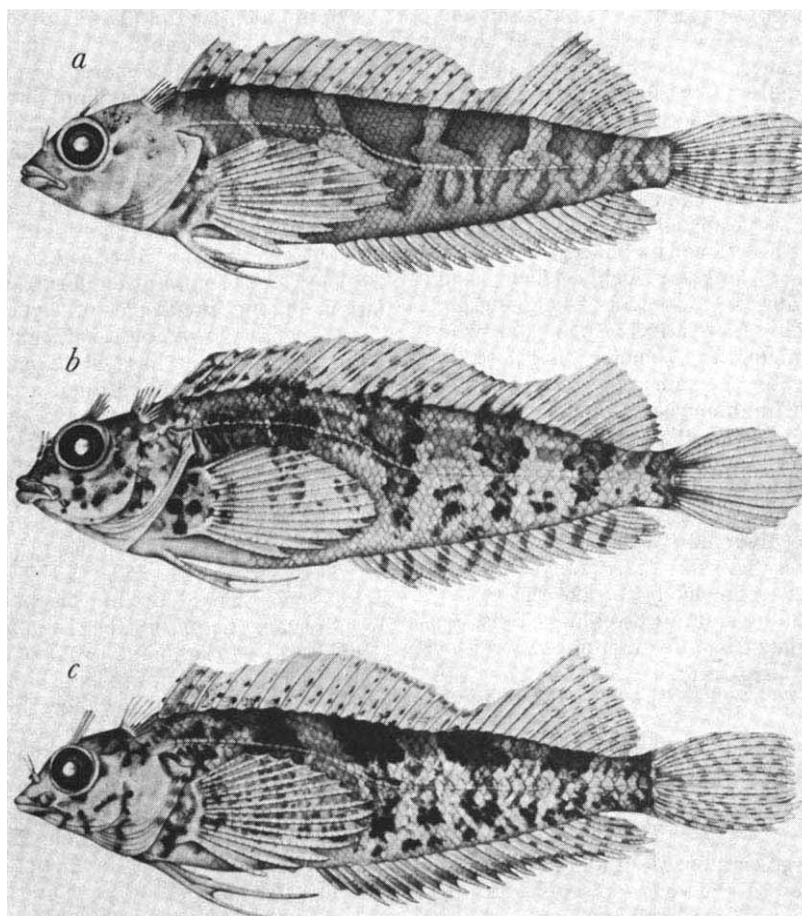
Some years ago, for example, Busse (*J. Math. Phys.*, **46**, 140; 1967) showed that two-dimensional convective rolls in a fluid layer between rigid stationary boundaries are only stable for Rayleigh numbers less than 13 times critical. At higher Rayleigh numbers, a second set of rolls develops at right angles to the original one, giving a rectangular pattern when viewed in plan (bimodal convection); and at Rayleigh numbers about 100 times critical, instability develops, giving a plan pattern which

resembles spokes radiating out of central upwellings or downwellings (multimodal convection). But in spite of this and other evidence, many workers have assumed stability for convective models of the upper mantle where the Rayleigh number is probably several hundred times critical. According to Richter and Parsons, for a model governed by nonlinear dynamics it is just not sufficient to find a solution satisfying the Rayleigh convection equations and the boundary conditions. The solution must also be shown to be physically possible; and only laboratory experiments can easily provide this critical information.

A good illustration of this point is provided by Richter's (*J. geophys. Res.*, **78**, 8735; 1973) own attempt to account for the pattern of oceanic heat flow. The high heat flow anomalies over active oceanic ridges can readily be explained in terms of large-scale convection. This is the well-known system in which mantle material rises at ridges, the seafloor spreads laterally and descends at oceanic trenches, and the cycle is completed by return fluid flow in the upper mantle. Much more difficult to understand, however, is the approximately constant and uniform heat flow through ocean floor older than a few tens of millions of years. Very little of the heat involved here can be produced in the oceanic crust itself which contains too few radioactive sources. Most of it (about $1 \mu\text{cal cm}^{-2}\text{s}^{-1}$) must therefore come up from the mantle. But this raises the problem of how the mantle can transport so much heat. Calculations based on the available knowledge of upper mantle composition and physical properties show that neither conduction nor radiation is sufficient.

To overcome this difficulty, Richter proposed the existence of small-scale convection beneath the plates—convective cells having a horizontal scale comparable with the depth of the fluid flow region but smaller than the plate dimensions which characterise the horizontal segments of large-scale convection. In other words, he envisaged two coexisting horizontal scales of motion in the upper mantle (the vertical scales are the same)—one required by the moving plates and one by heat flow through old ocean floor. The principal result to emerge from analytical and numerical studies of such a system was that the large-scale convection suppresses small-scale convective rolls having axes perpendicular to the large-scale flow (transverse rolls) and, in time, replaces them with rolls having axes in the direction of large-scale flow (longitudinal rolls). Unfortunately, however, this analysis was strictly valid only for Rayleigh numbers slightly higher than critical. So does it, or does

Same species, different profiles



Malacoctenus triangulatus females. *a*, from Hog Island, Bahamas; *b*, from Trinidad; *c*, from Brazil. Drawn by J. R. Schroeder; from *Smithsonian Contributions to Zoology* no. 200 by Victor G. Springer and Martin F. Gomon; Smithsonian Institution, 1975.

it not, also apply to Rayleigh numbers more typical of the mantle?

This is where laboratory work is crucial; and the results of a series of experiments designed to study the interaction of small-scale and large-scale convection have now been reported by Richter and Parsons. Briefly, these are that irrespective of the initial stable state of small-scale convection (transverse rolls, bimodal convection or multimodal convection, depending on Rayleigh number over the range 3.0×10^3 – 6.8×10^5), the imposition of large-scale convection ultimately changes the form of the small-scale flow to longitudinal rolls. The experimental data thus both demonstrate the plausibility of two-scale convection and confirm that the earlier theoretical results also apply at high Rayleigh numbers.

They also enable a time scale to be applied to the development of the longitudinal rolls under different conditions. Assuming that the Rayleigh

number of the sub-oceanic upper mantle approaches 10^6 and that convection extends down to the 650-km seismic discontinuity (assumptions which Richter and Parsons attempt to justify in detail), the experiments suggest that where the plate velocity is 10 cm yr^{-1} the small-scale convection should become aligned as longitudinal rolls within 20–50 Myr. On the other hand, for a plate velocity of 2 cm yr^{-1} the time scale increases to several hundred million years. Taking into account typical lengths of time between major changes in plate motion, these results suggest that longitudinal rolls within the small-scale convection system should only develop where rates of plate motion are high.

Richter and Parsons have thus combined theory, experiment and some observation (for example, of heat flow) to produce an internally consistent two-scale convection model. But can these ideas be tested, or are they destined to remain unprovable hypotheses? The

problem here is the shortage of observational constraints that can be related directly to convection rather than just to the rigidity of the plates. Two possibilities suggested by Richter and Parsons, however, are free-air gravity anomalies and residual elevation anomalies (differences between measured ocean floor depths and the corresponding depths to be expected as the result of the cooling of the lithosphere as it moves away from the ridge). As far as the roll-like pattern of convection is concerned, the best place to look for such evidence would probably be the Pacific where the plate velocity is relatively high and where no significant change in spreading direction has apparently occurred for 40–45 million years. But the region in which anomalies have been most closely observed so far is the North Atlantic where the slower spreading rate makes roll-like features less likely to be observed (nor are they) and thus where the small-scale convection pattern is more likely to take the form of upwelling and downwelling spouts. This mismatch is unfortunate, but with luck only temporary.

Peanuts and cancer

from Arie J. Zuckerman

THE geographical pathology of primary cancer of the liver presents intriguing features. Liver cell cancer is rare in North America, Europe, the Soviet Union and Australia and it seems to be relatively infrequent in Central and South America. In contrast, hepatocellular carcinoma is common in many communities in Africa and South-East Asia and probably less so in Japan. In some parts of the tropics, liver cell cancer is the commonest type of cancer in adult males and it is often, though not always, found in patients with cirrhosis. Epidemiological and experimental evidence is accumulating to implicate natural hepatocarcinogens as contaminants in foodstuffs and hepatitis B virus in the aetiology of primary liver cancer.

The carcinogenic potential of a number of mycotoxins and pyrrolizidine alkaloids is now well established in experimental animals. The aflatoxins form a group of closely related substances produced by certain strains of moulds of *Aspergillus flavus-oryzae*. These substances have been shown to have marked toxic and carcinogenic effects on the liver and other tissues of experimental animals. The cytotoxicity of aflatoxins on human embryo liver cells in cultures has also been demonstrated; aflatoxin inhibited DNA, RNA and protein synthesis in such cultures (Zuckerman *et al.*, *Br. J. exp.*

Path., **49**, 33; 1968). The order of toxicity of aflatoxin B₁ and G₁ in human liver cells was similar to the order of carcinogenicity in rats.

The mould *Aspergillus flavus* readily grows on groundnuts (peanuts) and grains when they are stored traditionally in the open, in hot, humid conditions. Indeed approximate relationships have been reported between aflatoxin contamination of market food samples and the incidence of primary liver cancer in Uganda, Swaziland and Thailand. Peers and Linsell (*Br. J. Cancer*, **27**, 473; 1973) found a significant correlation in the incidence of liver cell cancer and the amount of aflatoxin actually consumed in a study carried out in three different altitude areas in the Muranga district of Kenya. In the low altitude area, below 5,250 foot above sea level, the incidence of liver cancer was greater and the aflatoxin levels in the food samples were higher. Van Rensburg and colleagues (*S. Afric. J. Med.*, **48**, 250a; 1974) have also shown that aflatoxin consumption by Africans living in the Inhambane district of Mozambique was remarkably high, correlating well with the high prevalence of liver cancer in that area. Peanuts and, in some areas of Africa, maize form the staple diet of Africans and the consumption of food contaminated with fungal metabolic products is likely to be high. Yet it would be prudent to note that the quantity of aflatoxin that would need to be ingested by an individual in the form of contaminated peanuts, assuming that the dose is similar to that needed to cause experimental necrosis of the liver seen in animals, is in gross excess of that likely to be consumed, amounting to several kilograms per day (Higginson, *Tumours of the Liver*, Pack and Islami; Berlin, 1970).

The apparently high incidence of infection with hepatitis B virus in chronic liver disease and especially macronodular cirrhosis and primary liver cancer in many regions in Africa and South-East Asia also implies the possibility of some form of an aetiological association. That a strong correlation has been found in some studies cannot be denied, but there are also a number of conflicting reports from different regions. The most recent study by Bagshawe and her colleagues in Kenya (*Br. J. Cancer*, **31**, 581; 1975) revealed that in the Muranga district (see above) no significant differences were found in the prevalence of hepatitis B surface antigen (3.6%) between the low altitude areas, with a relatively high incidence of primary liver cancer, and the high altitude area (2.7%) with a low incidence of the tumour.

Liver cell cancer is probably the cumulative result of many factors, and the roles of viral and parasitic infec-

tion, chemical carcinogens, nutritional and other environmental factors remain to be determined.

QSOs still problematical

from R. F. Carswell

THE origin of absorption lines in some QSOs remains something of a puzzle. Perhaps the only thing that is clear is that there are absorbing clouds some distance from the QSO, which have redshifts which are usually less than that of the emitting object itself. The velocity differences are usually less than about one-tenth the speed of light, but in one well established case the velocity is about half the speed of light. Even so, the redshifts of the QSOs in which these absorptions are seen are so high that, even with these large velocities relative to the QSO, the clouds are still receding from us. Study of the absorption line regions is clearly important, since if they are intervening galaxies then they can give us some idea of the nature of galaxies during the early Universe, and if they are intrinsic to the QSOs then they must be telling us something about the properties of the QSOs themselves. There are a number of problems in interpreting the observational data however, which leave the situation unclear.

The first major problem is that of identifying the redshifts of the absorption clouds, given a spectrum which may contain anything up to 200–300 absorption lines. A single QSO may have many absorbing clouds, at many very different redshifts, each with differing excitation properties so that it is not always possible to say definitely what lines, or redshift systems, must be present. What is usually done in the case of a newly discovered absorption line QSO is to try all reasonable candidate lines at all possible redshifts, and see if any physically consistent, and statistically significant, absorption systems are present. Often this is done on a fairly subjective basis, but a computer program developed by Aaronson, McKee and Weisheit (*Astrophys. J.*, **198**, 13; 1975) may help bring a consistent approach to the subject. The authors have analysed all previously published data on absorption line QSOs looking for candidate absorption systems using a coherent set of atomic physics and ionisation equilibrium criteria, and checked the probability of finding such a system by chance. They show that the number of well-established systems is in fact much smaller than had previously been believed, notably in very absorp-

tion rich spectra such as PHL957 and 4C05.34 where there is a high probability of chance identifications. This still leaves a large number of unidentified lines, which may be due to optically thin clouds with too few absorption lines to be sure of their reality in individual cases, but which together account for most of the features in the spectrum.

Aaronson *et al.* also examine the question as to whether or not the probable absorption redshifts they have are likely to have arisen from intervening galaxies or matter ejected from the QSO. Perhaps not surprisingly, with only eight absorption line QSOs for which good spectral material is available, it seems that the available data are consistent with either picture, though they suggest that the intrinsic hypothesis might be slightly favoured. To verify this we must await additional material on new absorption line QSOs.

The question as to whether or not there are intervening galaxies between us and some QSOs depends to some extent on how distant the QSOs are, and this brings us to consideration of the nature of the redshift. It is generally believed that the high redshifts observed in QSOs are a result of nothing more than the general expansion of the Universe that seems well established from studies of normal galaxies at much lower redshifts. High redshifts are seen in QSOs only because they are intrinsically bright objects and can therefore be seen to much greater distances. But in spite of the wide acceptance of this view, some astronomers contend that there is really not sufficient evidence to say that there may not be some other explanation, for all or part of the high QSO redshifts, perhaps involving some physical principles of which we have no knowledge at present.

Given that we have no model of QSO behaviour under such circumstances, such a hypothesis is very difficult to test, but some headway can be made if we believe that some QSOs are physically associated with, for example, galaxies of much lower redshift. If this is true, then there could be an excess of QSOs near bright galaxies compared with the numbers we would expect on the basis of estimates of the number density of QSOs in the sky that have been made by Sandage and Luyten (*Astrophys. J.*, **155**, 913; 1968) and others. A few years ago Burbidge, Burbidge, Solomon and Strittmatter (*Astrophys. J.*, **170**, 233; 1971) found that in fact there were more QSOs identified from the 3C (third Cambridge) radio catalogue lying within 7' of bright galaxies than could be due to chance, and subsequently another 3C QSO was found close to such a galaxy. This was fairly

strong evidence, on the face of it, that QSOs had some intrinsic redshift. Subsequent studies of identifications from other radio catalogues did not show the same behaviour, though, and so after a while the result looked more as if it could have been due to chance apparent associations.

Recently, however, Arp, Baldwin and Wampler (*Astrophys. J.*, **198**, L3; 1975) have found two new radio-quiet QSOs near bright galaxies, and so now there are a total of four such known. On the basis of the estimate of the number of QSOs brighter than 19 mag the areas around several thousand galaxies would have to be searched to find four QSOs if they are randomly distributed, while the number of such searches made has been estimated by the authors to be about twelve. This suggests, then, either the estimates of numbers of radio-quiet QSOs are far too low or the associations are likely to be real. There are well-known dangers in drawing conclusions from statistics performed after the event, however carefully the work is done, so it is to be hoped that further searches in neighbourhoods of galaxies will be performed to see if the results are confirmed for a new sample.

Starting at the end

from R. Kamen

An EMBO-Inserm workshop on the *in vitro* transcription and translation of viral genomes was held on July 15–18 at Grignon, near Paris.

THE RNAs of picornaviruses such as polio and EMC have long been the paradigms for eukaryotic polycistronic messenger RNAs. These large RNAs encode a single nascent polypeptide which is cleaved to generate stable viral polypeptides, prompting the general hypothesis that eukaryotic mRNAs do not contain functional internal signals for the initiation of protein synthesis. The recent development of various plant and animal systems for *in vitro* protein synthesis and their successful application to the translation of a wide variety of viral messengers has provided a wealth of data generally supporting this hypothesis. The picornaviruses, however, now appear as one special case of a more general mechanism involving mRNA as well as protein processing. The results obtained in several laboratories, discussed at the Grignon workshop, fit a common pattern and experiments presented on the role of 5' terminal 'capping' (see *Nature*, **255**, 9; 1975) in translation may explain why eukaryotic ribosomes start at the ends of mRNA molecules.



A hundred years ago

A RETURN has been presented to Parliament giving a statement of all the weather telegrams issued by the Meteorological Office, and also of all the storms experienced on the coasts of the British Islands during 1874, from which it appears that of the warnings issued, 78.2 per cent. were justified by subsequent gales or strong winds, and that 16.4 per cent. were not justified by the subsequent weather. This percentage of success in the warnings issued, which is slightly in excess of the last two years' of Fitzroy's management, considerably in excess of 1870 and 1871, and about equal to the results for 1872 and 1873, is perhaps as good as may reasonably be expected until the system be further extended and developed. from *Nature*, **12**, 319; August 17, 1875

The process common to many of the viral systems discussed is the following: the 5' terminal portion of a viral polycistronic mRNA functions efficiently in encoding a viral protein or polyprotein, whereas the 3' terminal region remains largely silent even though it contains the sequences which encode a further viral polypeptide. A smaller viral mRNA, comprising the 3' terminal cistron, must be generated (either by RNA cleavage or by selective transcription) to activate translation of the second protein. P. Kaesberg's (University of Wisconsin) results on brome mosaic virus (BMV) well illustrate this mechanism. BMV is a multicomponent plant virus comprising three distinct virions containing four different RNAs. RNAs 1 and 2 each have their own particles, while RNA 3 (0.7×10^6 daltons) and RNA 4 (0.3×10^6 daltons) share the third type of particle. When translated in the wheat germ system, RNAs 1 and 2 prove to be monocistronic messengers coding for large proteins of size consistent with their RNA molecular weights; thus the proximity of the initiation point to a 5' end is assured by the genome segmentation. RNA 3, however, includes at its 3' end all of the sequences of RNA 4. When translated *in vitro*, RNA 3 encodes one major polypeptide (called 3a) unrelated to the BMV coat protein, while RNA 4 is an efficient messenger for the coat protein. Thus RNA 3 contains one active 5' proximal start signal and a second largely inactive internal start signal which is unmasked in the generation of RNA 4. Competition studies showed that the second start in its

active state, has in fact higher affinity for ribosomes than the start at the 5' end of RNA 3. Two alternative explanations for the dramatic alteration in efficiency of this initiation site between RNAs 3 and 4 were discussed: either the structure of RNA 3 blocks internal initiation, or a modification at the 5' end of RNA 4 is involved in ribosome binding. Isolation and sequencing of a fragment of RNA 4 which specifically binds to ribosomes led Kaesberg to opt tentatively for the second alternative: the sequence is m⁷GpppGUAUUAUAAUG . . . followed by the codons for the first ten amino acids of the coat protein. A. J. Shatkin (Roche, New Jersey) pointed out in discussion that the UAAU tetranucleotide of this sequence is complementary to the common 3' end of eukaryotic 18S rRNA recently determined by Steitz, Shein and Delgano. Thus the mechanism of ribosome recognition may be in part similar to that used in prokaryotes. Results presented by M. N. Thang (Institut Biologie Physico-Chimique, Paris) and by L. van Vloten-Doting (State University Leiden), obtained with the small RNAs of the related alfalfa mosaic virus, although more preliminary, concurred with Kaesberg's conclusions on BMV.

T. Hunt (University of Cambridge) discussed the *in vitro* translation of tobacco mosaic virus (TMV) RNA. He and his colleagues find that the 2.2×10^6 dalton genomic RNA encodes overlapping 160,000 and 140,000 dalton proteins, but no coat protein. A small viral RNA (0.25×10^6) found in infected leaves, comprising the 3' end of the total RNA, is a very efficient messenger for TMV coat protein in several *in vitro* systems. Among the plus-stranded RNA animal viruses, two groups—I. Kennedy (University of Warwick), and L. Kääriäinen (University of Helsinki)—reported experiments with Semliki Forest virus consistent with the notion that the 42S genomic RNA encodes large nonstructural proteins while the 26S viral mRNA found in infected cells (comprising the 3' terminal third of the 42S) is the mRNA encoding a different single polypeptide which is efficiently processed *in vitro* into the four viral structural proteins.

The mechanism also functions in DNA animal viruses. A. Smith (ICRF, London) and C. Prives (Weizmann Institute, Rehovot) agreed that the 19S late messenger either of SV40 or polyoma virus encodes the minor virion structural protein(s), but is an inefficient messenger for the major capsid protein (VP1). The 16S late messenger, which at least in the case of polyoma virus comprises the 3' terminal half of the 19S mRNA, is the messenger for VP1 synthesis *in vitro*. Y. Aloni

recently reported at the European Tumour Virus Group meeting that the 19S SV40 mRNA is cleaved to 16S in the cytoplasm, and several groups have found that it has an m⁷G capped 5' end.

Evidence implicating 5' terminal methylation in ribosome recognition was presented for VSV RNA by H. P. Ghosh (McMaster University, Ontario) and in detail for several messengers by Shatkin. The latter reiterated his published evidence that non-methylated reovirus or VSV mRNA functions poorly in wheat germ or mouse L cell *in vitro* systems, but can be stimulated by *in vitro* methylation in the presence of S-adenosylmethionine in either system. He also showed that chemical

removal of the terminal m⁷G from these viral messengers or from rabbit globin mRNA destroys messenger activity. Most interesting were direct experiments on messenger binding: the 7mG of reo messenger is required for binding to wheat germ ribosomes. A 35-nucleotide long fragment of reo messenger containing the 7mG 5' end, which rebinds to ribosomes, was isolated from sparsomycin initiation complexes after nuclease digestion. 7mG has also been found in the globin mRNA ribosome binding site. Finally, Shatkin described experiments in which the 7mGpppG^m 5' terminal dinucleotide of Reo virus was extended with various mixed or homopolymers and assayed for ribosome binding. Only the

New blood parasites for old

from F. E. G. Cox

THE malaria parasites belong to the protozoan sub-order Haemosporina which also contains a number of other blood parasites. From the earliest days of parasitology these have received considerable attention and it comes as something of a surprise to discover that until recently a whole new family has existed unrecognised in South American lizards (Lainson *et al.*, *Int. J. Parasit.*, **1**, 241; 1971; *Parasitology*, **68**, 117; 1974; *Parasitology*, **70**, 119; 1975). This family is the Garniidae and its importance has grown since its discovery in 1971 partly because it looks as if it is going to contain a large number of species and partly because of the implications of the various life cycles which its members undergo. The interpretation of these life cycles throw doubts on current ideas regarding the nature of reptilian malarias and on the evolution of the malaria parasites as a whole. So far, two genera have been described, *Garnia* with six species and *Fallisia* with four.

All the other members of the Haemosporina undergo asexual division, or schizogony, in fixed cells and the malaria parasites, in addition, multiply in circulating blood cells. In the Garniidae, however, schizogony occurs only in blood cells: in red cells in *Garnia* and white cells in *Fallisia*. Some species of *Fallisia* occur in neutrophils and thrombocytes, cells which have not been utilised by any other parasites. Since the discovery of the exoerythrocytic stages of the malaria parasites it has been widely accepted that schizogony in the blood evolved secondarily but this belief will now

have to be reassessed. In addition, all the descriptions of malaria parasites of lizards will have to be re-examined as Lainson and his colleagues have found that multiple infections with members of the Garniidae and with true pigmented malaria parasites are common and this may well have confused the original descriptions of different species. Telford (*Int. J. Parasit.*, **3**, 829; 1973) has claimed that some members of the Plasmodiidae, the malaria parasites, are not pigmented but the recent work indicates that there is no need to redefine this family in order to include non-pigmented forms.

While new blood parasites are being discovered in lizards it is likely that some widely accepted species (and even genera) in birds will have to disappear. Box (*J. Protozool.*, **22**, 165; 1975) has found that infections with the intestinal eimerian *Isospora serini* in canaries results in the appearance of extra-intestinal stages in blood monocytes, something that does not happen with another species *I. canaria*. Box notes the similarities between these extra-intestinal stages of *Isospora* and *Lankesterella* spp. and other eimerian parasites which occur in blood cells. This is an extension of the discovery a few years ago that *Toxoplasma gondii* in many mammals is an aberrant *Isospora* sp. of cats.

The status of all non-pigmented blood parasites will have to be looked at critically in the light of the separate studies of Box and Lainson and his colleagues and the parasites of mammals cannot be allowed to escape the scrutiny.

m7GpppG^mpoly(A,U) derivative, which should contain molecules with the . . . UAAU . . . sequence complementary to the 3' end of 18S ribosomal RNA, showed significant interaction with 80S ribosomes.

Several observations must be mentioned which are in apparent conflict with the appealing idea that eukaryotic ribosomes only start at ends because they interact with 7mG. Hunt mentioned evidence from TMV and M. Pranger (University of Utrecht) and Kaariainen gave evidence from Semliki Forest virus, that there is more than one initiation site in the 5' terminal portion of their respective mRNAs. R. Gesteland (Cold Spring Harbor), in discussing elegant experiments showing that yeast suppressor tRNAs work in mammalian cell-free systems, reconfirmed the observation that an internal cistron of bacteriophage Q β RNA is translated accurately by eukaryotic ribosomes and initiation factors. Moreover, to return finally to the picornaviruses, these RNAs can be translated *in vitro* although, as discussed by P. Fellner (Searle, High Wycombe), 7mG cannot be detected at their 5' ends.

Insectivorous grouse

from our Animal Ecology Correspondent

THE traditional management of heather moorland for red grouse production centres on regular burning of the vegetation so as to encourage sprouting of new green shoots. Such new growth is rich in nitrogen and phosphorus, elements now known to be correlated with performance of grouse populations (Moss, *J. Anim. Ecol.*, **38**, 103; 1969). The practice of burning seems right and proper since heather forms the bulk of the diet of adult grouse. But most species of animals are opportunist feeders—the grouse is no exception—and adherence to too rigid a management creed may deny some moors the extra grouse they could produce.

Different grouse moors do not have identical vegetation. About two-thirds of the total acreage in Britain is low lying and fairly dry. The remaining one-third lies at high altitude and the high annual rainfall it sustains keeps its deep peaty surface waterlogged almost the whole year through. Recent studies by Butterfield and Coulson on one such blanket bog moor reveal that adult grouse supplement their diet with quite considerable amounts of insect food (*J. Anim. Ecol.*, **44**, 601; 1975). The habitat is just right for craneflies, chiefly *Tipula subnodicornis* and *Molophilus ater*, and at the peak of emergence their densities may reach 15 m⁻²

and 400 m⁻² respectively. Adult male craneflies do not fly strongly and the females have no wings at all. They represent to the grouse an additional source of food almost as easy to harvest as the heather tips upon which they spend most of their adult lives. That adult grouse can develop a specific search image for craneflies is clear from the observation that one grouse shot in October 1970 had the remains of 495 adults of the rare *T. gimmerthali* in its stomach. This is more than the total number of individuals caught by British entomologists since the end of the nineteenth century.

Observations during May, June and July of fresh droppings taken from established grouse territories on damp moor revealed that the percentage containing insect material varied from 98% at the time of maximum emergence to 8% at the end of the hatching season. Most droppings contained plant material but in a small number the amount fell to less than a half of the total contents. Both species of cranefly were eaten by adult grouse, but chicks took considerably more of the small *M. ater* than they did of the larger *T. subnodicornis*. For adult grouse the reverse was true. By contrast, droppings from a dry moor were almost totally devoid of insect remains.

What is of great interest is that weight for weight, *T. subnodicornis* contains nine times more nitrogen, seven times more phosphorus, six times more sodium and twice as much potassium as heather shoots. *M. ater* is richer, too, in these elements but less so than its larger relative. The importance of these elements in grouse nutrition is shown by the choice by breeding grouse of growing tips of heather which contain high levels of nitrogen and phosphorus (Moss, *J. Anim. Ecol.*, **41**, 411; 1972). Moss, Watson and Parr confirmed that maternal nutrition did affect breeding success but what was important was the length of time during which heather growth was possible prior to laying (*J. Anim. Ecol.*, **44**, 233; 1975). Management plans for grouse moors are aimed at creating conditions for maximum breeding success and standing crop of grouse. On wet moors craneflies are not available until after egg laying and so cannot directly influence pre-laying nutrition. Their peak in abundance comes shortly after clutches are completed when, it could be argued, female grouse are in a state of nutrient depletion. By offering an easily gathered, rich food source at this time, they could play a vital part in the maintenance of grouse populations. A full-scale investigation of the relationship between grouse and insects seems now to be overdue, for if these preliminary findings are generally sub-

stantiated the future management of the wet third of Britain's grouse habitat may well reflect the need to maintain suitable cranefly sites.

Tropomyosin in transition

from E. J. O'Brien

IN a resting muscle the level of Ca²⁺ is very low and interaction between the thick, myosin-containing and the thin, actin-containing filaments is inhibited. When the muscle is stimulated, Ca²⁺ is released from the sarcoplasmic reticulum so that its concentration around the filaments rises sharply. The inhibition is then removed and myosin and actin can interact, split ATP and produce contraction. The means by which inhibition and its removal are obtained has been extensively investigated, and, in the main, attention has been directed at the proteins troponin and tropomyosin which are combined with actin in the thin filaments of many types of muscle.

In the electron microscope actin resembles two strings of beads twisted slowly around each other. Much more difficult to see, because of its narrow width (2 nm), is tropomyosin, but from diffraction and other studies of the thin filament, it has been shown to lie end-to-end in each of the two grooves between the actin strings. Attached at intervals of about 40 nm along the actin-tropomyosin complex is troponin, which has three components: one, TN-T, which binds to tropomyosin, one, TN-C which binds Ca²⁺ and one, TN-I, which is responsible for inhibition.

With TN-C removed the thin filament is not Ca²⁺-sensitive and remains permanently 'switched off', as in a resting muscle. Without any of the troponin components the actin-tropomyosin complex is also not Ca²⁺-sensitive, but in this case it stimulates the splitting of ATP by myosin, that is, it is 'switched on'. Wakabayashi, Huxley, Amos and Klug (*J. molec. Biol.*, **93**, 477; 1975) have studied the nature of the structural change involved in the on-off transition. Since the required structural detail is at the limit of resolution provided by negatively stained material, their analysis has relied heavily on the use of sophisticated computer processing of electron micrographs. This involves densitometry of the micrographs to produce a digital scan, and from this, and a knowledge of actin's helical symmetry, three-dimensional reconstructions of the filaments can be obtained.

Wakabayashi *et al.* prepared fila-

ments of actin+tropomyosin and of actin + tropomyosin + TN-T + TN-I and formed them into paracrystals by the addition of Mg^{2+} . From the digital scans of micrographs of the paracrystals they selected one filament at a time and reconstructed it in three dimensions. From many such reconstructions the best averages were taken and the two types of filament compared. In the first type of filament the tropomyosin may be clearly distinguished running near the centre of each of the grooves between actin strands. When the TN-T and TN-I are added, however, the tropomyosin is displaced through about 1 nm and becomes much more closely integrated with each actin strand.

From observations of changes in the X-ray diffraction pattern of living muscle at rest and during contraction, several authors have proposed that tropomyosin moves in the actin grooves when the muscle is stimulated. This leads naturally to the proposal that inhibition is caused by blocking by tropomyosin of the sites of actin that interact with myosin. On stimulation of the muscle, increased Ca^{2+} concentration is thought to cause a conformational change in troponin with a consequent displacement of tropomyosin towards the centre of each actin groove and removal of the inhibition. Since the distribution of troponin is discrete along the thin filament (one troponin for every seven actin subunits), whereas that of tropomyosin is continuous, the function of the latter is both to mediate and to amplify the effect of the former.

Because only the amplitudes and not the phases of reflections in the X-ray diffraction patterns are recorded, the tropomyosin movement model cannot be substantiated from the whole-muscle studies alone. This is where the electron microscope work proves so useful. The direct determination by Wakabayashi *et al.* of a tropomyosin shift under the influence of TN-I is therefore an important advance in understanding thin filament control.

The simplicity of the steric blocking mechanism is appealing, but not necessarily convincing, and it may only be a part of a system which involves other subtler allosteric interactions. It has also still to be demonstrated that in the so-called inhibitory position, the tropomyosin does actually cover the actin site where myosin would attach. There is still work to be done then. Moreover, evidence has recently been accumulating for a Ca^{2+} -sensitive regulatory role in the thick as well as in the thin filaments of vertebrate muscles. The full picture is bound to be complex and even the distinction between the process and regulation of contraction may become less clear-cut.

Interferon at Warwick

from Mike Clemens

Interferon seems to be a popular subject this summer. The most recent of several meetings on it was an EMBO Workshop held at the University of Warwick on July 29-31 in which much new information concerning the induction, purification and mechanism of action of this anti-viral protein was presented.

It is well known that the synthesis of interferon can be induced in cells not only by intact viruses but also by synthetic double-stranded polynucleotides. But it is not at all clear whether the latter have to enter cells or act at the surface membrane. D. Hutchinson (University of Warwick) gave a critical account of the evidence in favour of the latter conclusion. In spite of the almost inevitable solubilisation of some double-stranded RNA which occurs when cells are incubated with this inducer covalently bound to inert supports, direct action at the cell surface seems likely. The immediately subsequent events are still a mystery but considerable progress has been made in identifying the messenger RNA for interferon in cells exposed to double-stranded RNA. P. Pitha (Johns Hopkins University) showed that poly(A)-containing RNA from induced human fibroblasts was translated both in mouse cell-free systems and in intact frog oocytes to give products with human interferon activity. No interferon mRNA could be detected in uninduced cells and it seems that regulation of interferon production may well occur at the transcriptional level. D. Burke (University of Warwick) presented evidence that the inhibitor of transcription camptothecin prevents induction of interferon by virus but not by double-stranded RNA, but it is not yet known whether a virus-directed or a host-directed transcriptional event is involved in this effect.

The most controversial area of interferon research concerns its mechanism of action. This has recently been studied by M. Revel (Yale University) using somatic cell hybrids containing a limited number of human chromosomes. The requirement for chromosome 21 for sensitivity of cells to human interferon is now thought to be because of a specific membrane receptor for interferon coded by this chromosome, rather than an intracellular 'anti-viral protein'. Studies with human fibroblasts monosomic or trisomic for chromosome 21 have confirmed this conclusion.

The events that occur between inter-

action of interferon with its receptor and inhibition of virus replication remain something of a mystery. There was general agreement that protein synthesis is a major target for interferon action but D. Metz (National Institute for Medical Research) produced convincing evidence that, in the case of monkey cells infected with SV40 virus, there is selective inhibition of viral RNA synthesis or enhanced nuclear turnover of viral transcripts after interferon treatment. The recently discovered process of methylation of the 5' ends of newly synthesised mRNA may also be a target for interferon action. P. Lengyel (Yale University) reported that the rate and extent of methylation of reovirus mRNAs in interferon-treated cell extracts was only half that seen in control extracts. However, A. Ball (University of Connecticut) stated that, in an interesting coupled transcription-translation system, methylation of newly synthesised vesicular stomatitis virus RNA is not impaired in the presence of extracts of interferon-treated chick embryo cells. In the same incubations translation of this RNA was severely inhibited compared to its translation in control cell extracts.

A possible clue to the mechanism of inhibition of translation came from the observation by I. M. Kerr's laboratory (NIMR) that interferon-treated L-cell extracts are more sensitive to inhibition by double-stranded RNA than control extracts. T. Hunt (University of Cambridge) described some recent experiments demonstrating that, in reticulocyte cell-free systems, double-stranded RNA causes the ATP-dependent phosphorylation of an initiation factor. However several groups have shown that polypeptide chain elongation as well as initiation is impaired by interferon treatment so that the situation is rather complex. Lengyel and Ball both stated that the established ability of tRNA to reverse the interferon-mediated inhibition of translation is not observed under certain conditions and the physiological relevance of this effect must therefore remain open to doubt. It would be a brave person who predicts, on the basis of present knowledge, just how interferon prevents viral replication within animal cells.

Correction

In the article by Donald Boulter "Breeding for Protein Yield and Quality" (*Nature*, 256, 168; 1975) there occurred the phrase "the long-assumed negative relationship between protein yield and grain yield". The assumed negative relationship was in fact between protein content and grain yield.

review article

Analogies between embryonic (T/t) antigens and adult major histocompatibility (H-2) antigens

Karen Artzt* & Dorothea Bennett*

Progress has been made in defining cell surface components specified by the T/t locus in the mouse, both in their expression on sperm and, in the one case studied, on normal embryonic cells and teratocarcinoma. Some striking analogies between this putative embryonic cell recognition system and the adult major histocompatibility complex are presented.

It is now generally accepted that cell surface structures have a critical role in cellular interactions during development and that they are genetically programmed. We are fortunate, therefore, to have available in the mouse an extensive set of mutations at the *T/t* locus that not only control specific points in embryonic development, but apparently operate through the specification of cell surface structures on embryonic cells and sperm. The serological identification and biochemical study of the products of *T/t* locus genes may offer some new insights into the genetic control and mechanism of development.

The *T/t* locus

The *T/t* complex is located on chromosome 17, fourteen crossover units to the left of *H-2*. It is marked by a class of semi-dominant *T* (Brachyury) mutations which, when heterozygous, cause a short tail and when homozygous are lethal^{1,2}. But more important, *T* mutations interact with recessive alleles at this locus to produce a tailless (*T/t*) phenotype; this interaction makes possible the detection of recessive *t* alleles that would otherwise go unnoticed.

A large number of different *t* mutants have been detected; as homozygotes some are embryonic lethals, some are semilethal (some, but not all, homozygotes of this class die), and many are viable. Here we will consider mainly the lethal ones. These lethal mutations are not laboratory curiosities since they exist as natural polymorphisms in wild populations of mice the world over, where they are maintained by a distortion of their rate of transmission through males (but not females). Better than 90%, instead of the expected mendelian 50%, of the progeny of a heterozygous male (*+/-t*) and a normal female (*+/+*) will carry the *t* allele³. Lethal *t* mutants also have the unique property of suppressing normal recombination, by some mechanism still obscure, along 14 units of chromosome between *T* and *H-2* (ref. 4, D.B., unpublished data and C. Hammerberg and J. Klein, personal communication). About one gamete in 10⁻³ does, however, carry a recombinant chromosome, as judged by the exchange of an outside marker. This kind of recombination event almost always generates a new and different *t* allele⁵, usually viable. Observation of these transitions is facilitated because two different *t* mutants complement one another, so that *t¹t²* animals survive and are normal in all respects except male fertility (for reviews see refs 3, 6 and 7).

The *T/t* locus clearly contains a set of genes of critical importance in early stages of embryonic development. We know that normal alleles are essential for normal development because homozygotes for any one of eleven different mutant alleles die at characteristic stages during embryogenesis, usually when a particular cell type proves incapable of maintaining itself or proceeding to the next step in differentiation. Morphological studies of affected homozygous embryos^{6,8} provide a dramatic picture of failure of cell-cell interactions, and suggest strongly that the initial defect can be associated with the cell membrane^{9,10}.

These observations led in 1970 to a search for membrane structures specified by the *T/t* locus. Since sperm cells are also affected by *t* mutations¹¹ and since they were readily obtainable in quantity, whereas young embryos were not, sperm were used initially to define *T/t* cell surface structures. Immunisation of mice with mutant (*T/t*) sperm and appropriate absorption of the resulting antisera with + sperm yielded specific antibodies that were demonstrated in complement-mediated cytotoxicity tests to be directed against the products of *T* and several different recessive *t* mutations^{12,13}. These initial studies and work in progress show: that codominantly specified *T/t* antigens exist on the sperm surface, that certain of the different *t* haplotypes produce unique specificities whereas others are cross reacting, that cross reacting antigens can in turn be resolved by absorption analysis into multiple specificities¹⁴, and finally, that some of these specificities are separated from one another by the rare recombination events that generate a new *t* allele from a pre-existing one (unpublished results).

Once it was established that mutations in the *T/t* complex were in fact associated with serologically detectable alterations of the sperm surface, the task was to validate our hypothesis that these antigens also appeared on specific groups of embryonic cells at particular stages of development. It is technically difficult to test this assumption using mutant embryos. We therefore turned to the study of teratoma cell lines established and characterised in the laboratory of F. Jacob, to test the more general hypothesis that embryonic cells can be characterised by their display of genetically controlled cell- and stage-specific antigens.

Teratoma system

Teratomas are tumours that develop spontaneously from germ cells, or from experimentally manipulated embryos, and represent a form of disorganised parthenogenesis that gives rise to derivatives of all three germ layers: ectoderm, mesoderm

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and endoderm¹⁵. Male mice of strain 129/Sv produce a high proportion of such tumours, and these tumours have been extensively studied both *in vivo* and *in vitro*¹⁶. An interesting and technically very helpful feature of teratoma cell culture lines is that different derivatives of the same tumour may form stable lines with different capacities for differentiation. For example, essentially pure cultures of the following types have been isolated. First, lines containing primitive teratocarcinoma (PTC) stem cells that are multipotential and capable of producing derivatives of all three germ layers. Second, lines containing differentiated teratocarcinoma (DTC) cells that are restricted to a particular pathway in development, and capable of producing only a limited array of differentiated cell types, for example yolk-sac carcinoma, or myoblasts. Third, lines containing PTC cells that have lost all capacity for differentiation and are nullipotent. Cultures of the last two kinds provide relatively homogeneous populations of embryonic cells that seem to be permanently trapped at specific developmental steps, and therefore can be used to attempt to identify cell- and stage-specific surface components.

The hypothesis was that cell surface structures concerned with early embryonic development appear and disappear long before immunological self tolerance, and therefore would behave as autoantigens¹⁷. (T/t antigens are on adult sperm but act as autoantigens to males because of their immunologically privileged location within the seminiferous tubules.)

The most simplified PTC cell line (F9) of the third category above was used to prepare syngeneic hyperimmune antisera against irradiated cells. This antiserum was assayed in complement-mediated cytotoxicity tests, and by absorption; it was found to react only with lines of teratoma which contained PTC cells, of either nullipotent or multipotential variety, but not with differentiated lines. The serum was also negative on all adult somatic cells tested, as well as a wide variety of tumour cells. Importantly, however, the anti-F9 serum reacted with sperm cells and normal cleavage stage embryos¹⁸; expression is low on one-celled embryos, increases to a maximum at about 8 cells, and then declines again (unpublished data). The exact timing of the disappearance of the antigen from the embryo is still under study, but it is not present on any differentiated cells that have been examined.

These results suggested that anti-F9 recognised a cell surface structure present on uncommitted cells and absent from differentiated cells. The antigen recognised was not a tumour antigen, and its normal tissue distribution was restricted to sperm and early embryos.

Relationship of F9 antigen to *T/t* locus

The general distribution of F9 parallels that postulated for *T/t* antigens. The parallel was quite precise with respect to a particular *t* lethal (*t*¹²) as *t*¹² homozygotes become abnormal and die at the morula stage. It seemed a promising possibility that the antigen detected on PTC-F9 cells, normal morulae and sperm, might be specified by the wild type allele of *t*¹², since of course, the genotype of the teratoma did not contain *t*¹², but rather (+) alleles at the *T/t* locus. Tests of this possibility were available, since the expression of *T/t* antigens on sperm is codominant¹³. Thus, if F9 were specified by the (+) allele of *t*¹², sperm from +*t*¹² males should carry only half as much F9 antigen as sperm from +/+ males.

The relationship of F9 to +*t*¹² was therefore tested by absorbing anti-F9 with known numbers of sperm from males heterozygous for *t*¹², and males that were either wild type or heterozygous for other *T/t* mutations. It was consistently found that sperm from males carrying *t*¹² were only half as effective as all other types in removing anti-F9 activity¹⁹. The relative paucity of F9 antigen on sperm from *t*¹² heterozygotes was confirmed with direct cytotoxicity tests of anti-F9 on sperm from various genotypes; *T/t*¹² sperm were only half as susceptible to complement dependent lysis as were sperm from *T/+* males or males carrying different later-acting *t* alleles (*T/t*⁰, *T/t*^{w5}, *T/t*⁹) (unpublished results). Thus, F9

antigen seems to be specified by the +*t*¹² gene.

To recapitulate, these experiments have been encouraging on three fronts: first, teratoma cells at a defined stage of differentiation have proved to be valid models for cells of the normal embryo; second, a genetically specified, stage-specific, embryonic cell-surface antigen (F9) has been defined; and third, the appearance of this antigen in the embryo has been shown to correlate temporally with the developmental function of the gene responsible for it. The functional role of this antigen in development is as yet unknown, but it may reasonably be speculated that it is concerned with cell-cell interactions.

Analogy between the *T/t* complex and MHC

Our own conceptual framework for some years has been that the *T/t* locus may represent an embryonic analogue of the adult major histocompatibility complex (MHC), and that both systems operate as mediators of cell-cell recognition; the former functioning in the embryo, the latter in the adult. The evidence in support of this hypothesis is as follows.

First, the *T/t* complex and the *H-2* complex of the mouse are linked on chromosome 17. Their linkage may not be entirely fortuitous. In the presence of most lethal and semi-lethal *t* alleles, recombination is suppressed between *t* and *H-2*, a distance of 14 cmorgans. This suppression is unexplained, but led Snell²⁰ as early as 1968 to propose that the two loci comprised a "super gene" acting as a functional unit for maintaining heterozygosity at *H-2* in feral populations of mice.

Second, each complex occupies a large region of chromosome. The *H-2* complex spans 0.4 cmorgans²¹; the *T* complex is not, as yet, measured because of the recombination suppression associated with *t* alleles; but this fact alone, and evidence that *t* mutation consists of several different functional elements separable by exceptional recombination, suggest that it also occupies a long region.

Third, both *T/t* and *H-2* specify codominant antigens on the cell surface which are complex and highly polymorphic. Each *H-2* haplotype of independent origin encodes on the average eight specificities²². Haplotypes of *t* mutants have three or four specificities; the total number currently defined is six, but this number rests on the analysis of only three cross reacting haplotypes¹⁴. Furthermore, since wild type specificities have not been taken into account at all, the six specificities so far defined probably represent only a fraction of the total.

Fourth, each complex locus has a minimum of two serologically detectable (SD) genes. The two SD regions of *H-2* are well documented²³. The *t* lethals have long been known to consist of at least two functionally different regions separable by crossingover, namely a factor responsible for lethality and a factor responsible for phenotypic interaction with *T*. This is made obvious by the generation of viable alleles by recombination²⁴.

We have studied several of these recombinant haplotypes serologically, and in every case direct cytotoxicity tests demonstrate cross reactivity with the parental haplotype. In the one case analysed by absorption, when antiserum specific for the parental lethal is thoroughly absorbed with the recombinant *t* viable sperm, it retains activity for the parental lethal and for other cross reactive lethal haplotypes. On the other hand, the absorbed antiserum is negative on other viable haplotypes independently generated from the same parental lethal haplotype. The serological data are consistent with the functional criteria mentioned above and suggest that the crossover haplotype has lost the unique specificities associated with lethality but retained other mutant specificities (unpublished).

Fifth, the developmental expression of *H-2* and *T/t* antigens seem to be reciprocal. *H-2* is present on all adult cells, with the apparent exception of sperm²⁵, but absent from the early embryo²⁶ and analogous cells such as F9 (refs 25 and 27). *T/t*, on the other hand, is absent from all adult cells except germ cells¹², but is present on embryonic cells¹⁹. *H-2* is first detectable in 7-9-d embryos²⁸, whereas *T/t* antigens associated

with lethal haplotypes are presumed to cease expression by 9 days of development. Furthermore, as *in vitro* populations of multipotential teratoma cells differentiate, detectable levels of F9 antigen fall, while H-2 shows a concomitant rise²⁸.

Sixth, both systems seem to have analogues in other species. MHC complexes like H-2 have been found in all mammals studied, as well as birds²¹. Likewise, antigens cross reacting with F9 (+*t*¹²) have been found on all mammalian sperm examined³⁰, including human sperm^{31,32}.

Finally, recent biochemical evidence suggests that the antigenic products of both *T/t* and H-2 are structurally similar. Lactoperoxidase radioiodination of cell surface components of lymphocytes, sperm and F9 cells, and immunoprecipitation with appropriate antisera, show not only that the molecular weight and subunit structure of the D and K products of H-2 and the F9 (+*t*¹²) antigen are identical, but also that +*t*¹², like H-2, is associated with a B-2-microglobulin-like moiety in the membrane²⁵. Similar results have been reported for the TL antigen^{33,34} which is closely linked to H-2D and apparently has a reciprocal interaction with it in the plasma membrane. Amino acid sequencing of +*t*¹² is now under way and together with sequence data for H-2, will prove or disprove the homology strongly suggested by these data.

The considerations above suggest that the *T/t* complex may be an evolutionary precursor of the MHC, or that both complexes originated in a common ancestral gene. An embryonic recognition mechanism of the type associated with *T/t* genes could have evolved with the metazoans at the stage when an immune system was not yet organised, indeed, not yet necessary. Later, with the advent of vertebrates and especially warm blooded animals, when protection from microorganisms became imperative, the duplication and specialisation of a complex genetic region already governing cell recognition may have been an economical way of providing genes for immuno-

logical recognition.

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articles

Genetic control of cell size at cell division in yeast

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A temperature-sensitive mutant strain of the fission yeast Schizosaccharomyces pombe has been isolated which divides at half the size of the wild type. Study of this strain suggests that there is a cell size control over DNA synthesis and a second control acting on nuclear division.

GROWING cells tend to maintain a constant size at division¹⁻³. This implies that there is coordination between cellular growth and cell division which results in a division occurring when the cell has grown to a particular size. The mechanism by which this is achieved remains obscure although there are various theoretical models which could account for the phenomenon⁴. A new approach to the problem would be the study of mutant strains in which the normal coordination between cellular growth and cell division is disrupted, resulting in the division of cells at different sizes. Such an approach in a simple eukaryote is described in this report.

A mutant strain of the fission yeast *Schizosaccharomyces*

pombe has been isolated which divides at half the wild-type size. Study of this strain demonstrates that its cell cycle is also profoundly altered compared with wild type. The timing of DNA synthesis in the cell cycle, the size at which cells undergo DNA synthesis, and the size at which cells undergo nuclear division are all different from wild type growing with the same generation time. These observations may be understood in terms of models of cell cycle control that propose a cell size control over DNA synthesis and a second control acting on nuclear division.

Cell division cycle

The cell division cycle of fission yeast has been extensively studied^{5,6}. Cell division is marked by the formation of a prominent cell plate across the middle of the cell, and separation of the two cells occurs a little later. The cell remains constant in diameter, growing only in length during the cell cycle. Cell length is therefore directly related to cell volume. As in higher eukaryotes the DNA-nuclear cycle can be divided into G₁, S, G₂ and M periods. The S period is very short and occurs at the beginning of the cell cycle

Table 1 Characteristics of wild type and mutant at 25 °C and 35 °C

Strain	Growth temperature (°C)	Generation time (min)	Cell volume at cell division (μm^3)		Cell volume at nuclear division (μm^3)		Macromolecular content per cell	
			Mean	s.d.	Mean	s.d.	Protein (pg/cell)	RNA (pg/cell)
Wild-type 972	25	228	129	9.1	121	8.4	12.4	2.54
	35	142	149	6.4	139	9.0	14.1	2.90
Mutant <i>cdc9-50</i>	25	232	109	8.8	98.0	7.9	9.66	2.26
	35	138	72.7	7.8	60.4	8.4	7.16	1.54

Cultures of the two strains were grown in a modified EMM 2 minimal medium⁵, with the sodium acetate and sodium dihydrogen orthophosphate replaced by 15 mM potassium hydrogen phthalate and 10 mM disodium hydrogen orthophosphate. Photographs of cells grown at 25 °C and 5 h after shift to 35 °C were taken using a $\times 16$ objective in a Zeiss photomicroscope under dark field optics. The length and diameter of 200 cells with cell plates were measured and their volume calculated. Protein and RNA content per cell, and generation times were determined in exponentially growing cultures as described in ref. 12. Between 5 and 10 determinations were made for each estimate of macromolecular content per cell and in no case did the standard error exceed 4% of the mean. Cells with dividing nuclei were detected by staining with Giemsa⁵. The volume of 200 such cells was calculated after correction for cell shrinkage on fixation and staining.

simultaneously with cell separation. A long G2 period follows culminating in nuclear division about three-quarters of the way through the cell cycle. There is then a short G1 period ending with the start of the S period of the next cell cycle.

Characterisation of mutant strain

The mutant strain *cdc9-50* was isolated from wild-type *S. pombe* (972h⁻ obtained from U. Leupold, Bern) after nitrosoguanidine mutagenesis (P. Nurse and B. Carter, unpublished). When grown at 35 °C, cells of strain *cdc9-50* divide at about half the volume of wild-type cells at that temperature (Table 1). The mutant phenotype is temperature-enhanced since at 25 °C *cdc9-50* divides at a cell volume only 15% smaller than wild type (Table 1). These differences are also reflected in the RNA and protein content per cell in exponentially growing cultures of the two strains at the two temperatures (Table 1). Thus *cdc9-50* divides at both a lower cell volume and a reduced macromolecular content compared with wild type. In spite of these differences *cdc9-50* has a similar generation time to wild type at both 25 °C and 35 °C (Table 1).

The mutant phenotype of reduced cell size at division segregated 2 : 2 in all of 27 asci dissected from a wild-type 975 h⁺ $\times$ *cdc9-50*h⁻ cross (P. Thuriaux, personal communication), demonstrating that it is caused by a mutation in a single nuclear gene.

Size control over nuclear division

Use was made of the temperature sensitivity of the mutant phenotype to determine the stage in the cell division cycle at which the coupling of cellular growth to cell division takes place. Exponentially growing cultures of *cdc9-50* and wild type at 25 °C were shifted to 35 °C. Cell number in the wild-type culture continued to increase for 50 min, stopped for about 30 min, and then gradually increased to the normal growth rate at 35 °C (Fig. 1). During the short plateau in cell numbers, RNA and protein continued to increase, consistent with the increase in cell volume at division that is observed at this time (Fig. 1). The temperature shift temporarily inhibits nuclear division, since for 25 min after the shift there is no further increase in the number of nuclei per ml of culture, and the normal rate of increase at 35 °C is only reached after a further 70 min (Fig. 1). Those cells in which nuclear division is temporarily inhibited are delayed from entering cell division, accounting for the observed short plateau and reduced rate of increase in cell number.

On shifting the culture of *cdc9-50* from 25 °C to 35 °C, cell number continued to increase for 50 min and then almost stopped, similar to the behaviour observed in wild

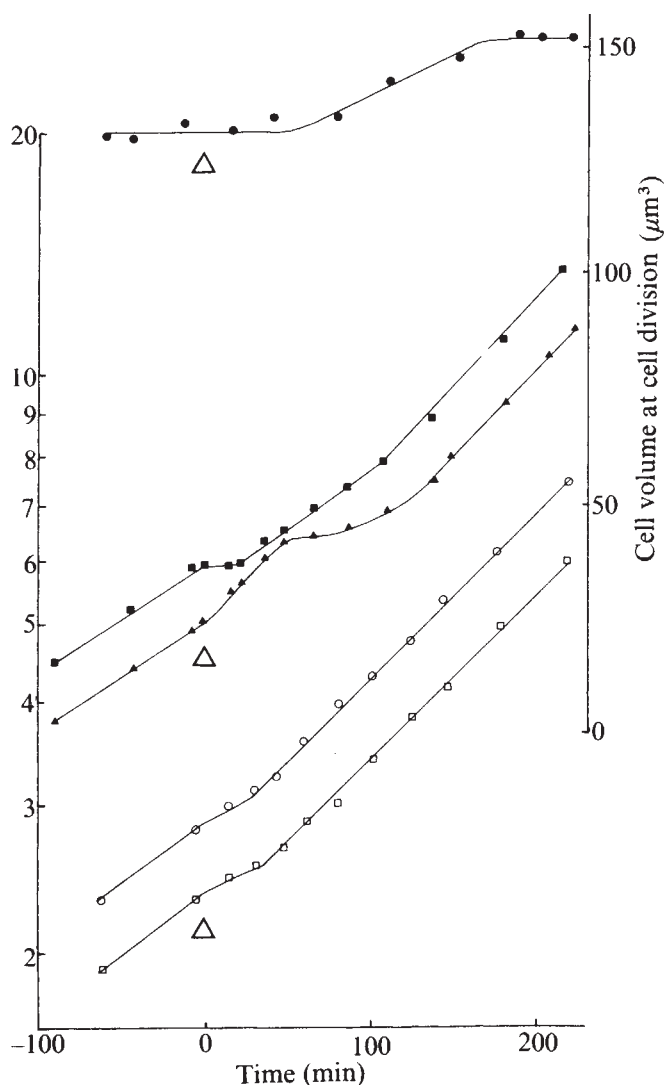


Fig. 1 Cell number, nuclei number, RNA, protein, and cell volume at division of wild-type 972 during the transition from exponential growth at 25 °C to 35 °C. A culture of 972 growing at 25 °C was shifted at 0 min to 35 °C, the culture reaching the higher temperature within 5 min of transfer. The time of transfer is marked by the large open triangles. Experimental parameters were determined as described in Table 1. The real values of experimental parameters per ml, equivalent to one unit on the arbitrary log scale, is given in brackets in the symbol key. ■, No. of nuclei ml⁻¹ (1×10^6); ▲, cell number ml⁻¹ (1×10^6); ○, protein ml⁻¹ (21.9 μg); □, RNA ml⁻¹ (5.46 μg); ●, cell volume at cell division.

type (Fig. 2). Unlike wild type, however, the plateau in cell number was very short (10–15 min) and was followed by a rapid rise rather faster than the usual rate of increase at 35 °C (Fig. 2). This rapid rise in cell number was preceded by a dramatic drop in cell volume at division (Fig. 2). RNA and protein continued to rise as in wild type (Fig. 2) and thus the RNA and protein content per cell dropped to the reduced level observed in exponential culture at 35 °C (Table 1). For 20 min after the temperature shift there was no increase in the number of nuclei per ml of culture showing an inhibition of nuclear division similar to wild type (Fig. 2). Thereafter there was a very rapid rise for a period of 40–50 min (Fig. 2), much faster than the normal rate of increase at 35 °C. This suggests that nuclear division is initiated in a large proportion of the population after a period of about 20 min at the higher temperature.

These data can be explained in terms of a size control over the initiation of nuclear division. In wild type at 25 °C the cell grows to a critical size and nuclear division is then initiated. On shift to 35 °C this control is modulated so that the cell grows to a larger size before nuclear division can take place. In *cdc9-50* the size control is altered so that at 35 °C nuclear division occurs in cells of a smaller size than wild type. On shift from 25 °C to 35 °C nuclear division is briefly inhibited by the same modulation that is observed in wild type. The mutant phenotype, however, rapidly expresses itself by reducing the cell size at which nuclear division is initiated. This results in nuclear division occurring immediately in that proportion of the cell population that were too small for initiation at 25 °C, but which were above the smaller critical size required at 35 °C. Thus cells that were too small to divide at 25 °C would be accelerated through the cell division cycle and would divide at the smaller size characteristic of growth at 35 °C.

If *cdc9-50* has an altered size control over the initiation of nuclear division, then nuclear division should occur in cells of a smaller size compared with wild type. When grown at 35 °C cells of *cdc9-50* containing dividing nuclei were about half the volume of similar cells of wild type (Table 1). At 25 °C cells of *cdc9-50* containing dividing nuclei were only about 20% smaller than wild type (Table 1). These relative differences are similar to the differences observed in cell volume at cell division for the two strains at the two temperatures. This would suggest that the size control over nuclear division determines cell size at cell division.

Timing of DNA synthesis during the cell cycle

The DNA content per cell in exponentially growing cultures is slightly reduced in *cdc9-50* at 35 °C compared with wild type (Table 2). This suggests that a greater proportion of cells have the unreplicated 1C content of DNA in *cdc9-50*, and hence DNA synthesis occurs a little later in the cell cycle than in wild type. This was checked by

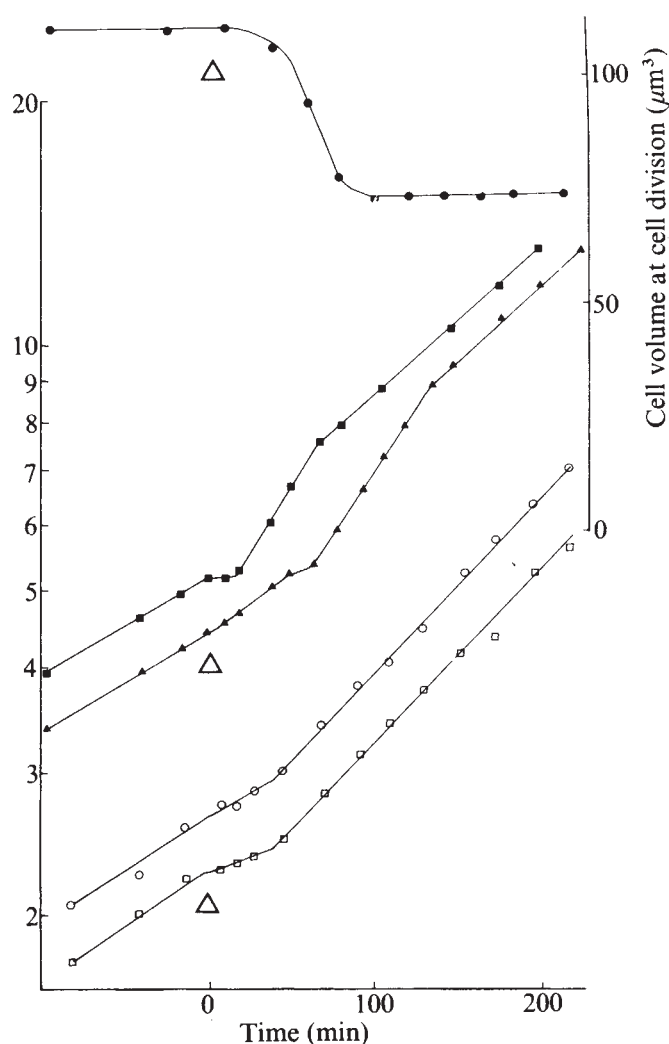


Fig. 2 Cell number, nuclei number, RNA, protein and cell volume at division of *cdc9-50* during the transition from exponential growth at 25 °C to 35 °C. All experimental details as for Fig. 1. ■, No. of nuclei ml⁻¹ (1×10^6); ▲, cell number ml⁻¹ (1×10^6); ○, protein ml⁻¹ (16.8 μg); □, RNA ml⁻¹ (4.58 μg); ●, cell volume at cell division.

measuring the increase in cell number in exponentially growing cultures after inhibiting DNA synthesis with hydroxyurea². Under these conditions only those cells which have completed DNA synthesis can undergo cell division. The cell number increase for *cdc9-50* was reduced compared with wild type (Table 2), again suggesting that DNA synthesis occurs later in the cell cycle in the mutant strain.

Table 2 Timing during the cell cycle and cell size at which DNA synthesis takes place in wild-type 972 and *cdc9-50* growing at 35 °C

Strain	DNA content per cell in exponential culture (fg per cell)		Cell number increase after inhibition of DNA synthesis	Fraction of a cell division cycle at which midpoint of rise in DNA occurred	Macromolecular content per cell at midpoint of rise in DNA			
	Mean	s.e.			Calculated Protein (pg per cell)	Calculated RNA (pg per cell)	Measured Protein (pg per cell)	Measured RNA (pg per cell)
Wild type 972	34.6	1.4	$\times 2.04$	0.00	10.2	2.09	12.0	2.61
Mutant <i>cdc9-50</i>	28.4	0.77	$\times 1.86$	0.29	6.29	1.35	5.87	1.37

Strains were grown at 35 °C as described in Table 1; DNA, protein and RNA content per cell were determined as described in ref. 12. Inhibition of DNA synthesis was achieved by the addition of 11 mM hydroxyurea. Three synchronous cultures were prepared as described in Fig. 3 and the mean fraction of the cell cycle at which the midpoint of rise in DNA occurred was determined (cell separation was taken as 0). The macromolecular content per cell at this time was measured, and was also calculated using the method described in ref. 8 and the data from Table 1.

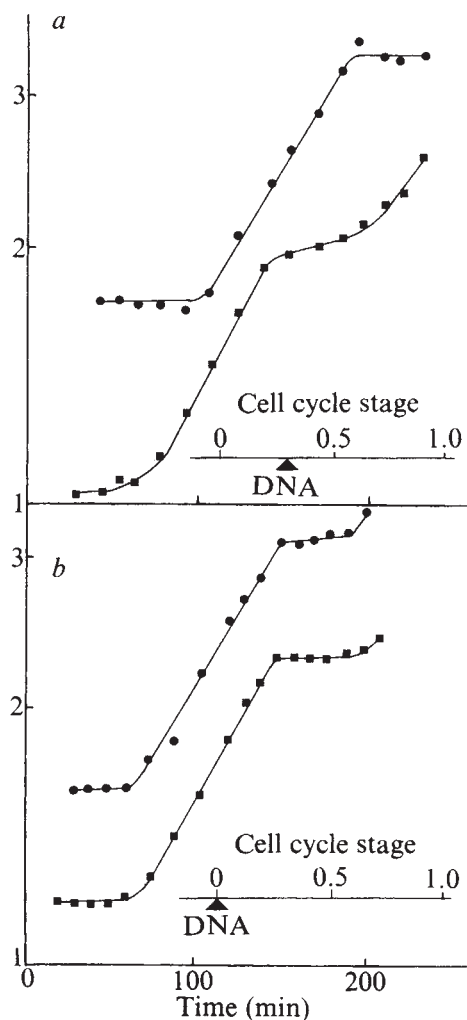


Fig. 3 DNA and cell number during synchronous cultures at 35 °C of *cdc9-50* (a) and wild-type 972 (b). Strains were grown at 35 °C as described in Table 1. Small cells were selected from these cultures by sedimentation rate centrifugation through a lactose gradient in a zonal rotor⁷. The small cells were used as an inoculum for synchronous cultures and DNA and cell number determined, as described in ref. 12. A cell cycle map is given for both cultures starting at cell separation and showing the time of the midpoint of the rise in DNA. The real values of the experimental parameters per ml, equivalent to one unit on the arbitrary log scale, is given in brackets in the symbol key. a, ●, DNA ml⁻¹ (8.63 ng); ■, cell number ml⁻¹ (4 × 10⁶). b, ●, DNA ml⁻¹ (7.47 ng); ■, cell number ml⁻¹ (3 × 10⁶).

These observations were confirmed by synchronous cultures of *cdc9-50* and wild type at 35 °C. Synchronous cultures were prepared by selection of small cells from an exponentially growing asynchronous population using sedimentation rate centrifugation through a lactose density gradient^{5,7}. In wild type the midpoint of the rise in DNA occurred at the beginning of the cell cycle simultaneously with cell separation (Fig. 3, Table 2). This timing is similar to that found in wild type at 32 °C (ref. 6). In *cdc9-50*, however, the midpoint of the rise in DNA occurred at 0.3 of a cell cycle, about 40 min later than cell separation (Fig. 3, Table 2). Thus in *cdc9-50* the G1 period is substantially lengthened at the expense of G2.

Knowledge of the timing of DNA synthesis during the cell cycle, and of the protein or RNA content per cell of an exponentially growing culture enables the protein or RNA content of cells undergoing DNA synthesis to be calculated⁸. Assuming protein and RNA to be accumulated exponentially during the cell cycle⁹, the protein and RNA content

of cells undergoing DNA synthesis has been calculated for 972 and *cdc9-50* at 35 °C (Table 2). It can be seen that the macromolecular content of these cells is much reduced in *cdc9-50* compared with wild type. This was confirmed by measuring the actual protein and RNA content of cells at the midpoint of the rise in DNA in the synchronous cultures (Table 2).

These results demonstrate that the mutant strain shows several differences to wild type. The timing of DNA synthesis in the cell cycle, the size at which cells undergo DNA synthesis, and the size at which cells undergo nuclear division are all different from wild type growing under identical conditions with the same generation time. Thus the single genetic lesion in *cdc9-50* has profoundly altered the controls acting on a number of events occurring during the cell division cycle.

Model of cell cycle controls

To explain the pleiotropic behaviour of *cdc9-50* and the maintenance of a constant cell size at division, it is necessary to invoke controls that act at both nuclear division and DNA synthesis. One such model would be that a cell size control acts over the initiation of nuclear division, and a second separate size control acts over an event required for initiation of DNA synthesis. The latter size control refers more precisely to a critical mass per genome which in wild-type cells is attained in the cell cycle previous to that in which DNA synthesis actually occurs. This is because the size control over nuclear division produces daughter cells that are already larger than the threshold size required to initiate DNA synthesis. Since nuclear division is required before DNA synthesis can take place (ref. 10 and my unpublished results using temperature-sensitive mutants defective in nuclear division), DNA synthesis cannot occur early in the previous cell cycle at the threshold cell size, but will be delayed until after nuclear division. Therefore the size control over DNA synthesis would not be expressed. In *cdc9-50* the genetic lesion reduces the cell size at which nuclear division occurs, but does not alter the size control over the initiation event required for DNA synthesis. In this situation the size control over nuclear division produces daughter cells smaller than the threshold size required to initiate DNA synthesis. The cell then has to grow for a further 0.3 of a cell cycle before it is large enough for DNA synthesis to take place. In wild type the size control over DNA synthesis may be expressed only when the cell is growing in adverse conditions. For example, on approaching stationary phase, yeast cells tend to divide at a smaller size^{5,11,12}, and so the size control over DNA synthesis would be expressed. The operation of the size control would then prevent a new DNA synthesis–nuclear division cycle being started, accounting for the observed accumulation of stationary phase yeast cells in the G1 period of the cell cycle^{11,12}.

It should be noted that nuclear division could seem to be under a size control if there was a timing mechanism maintaining a constant period between the initiation event required for DNA synthesis and nuclear division. If the genetic lesion in *cdc9-50* resulted in a shortened period compared with wild type, then nuclear division would be initiated earlier when the cell had grown less, and hence cell division would take place at a smaller size. In this situation which is directly analogous to that proposed for the control of the cell cycle in bacteria¹³, *cdc9-50* should be considered as altered in a timing mechanism rather than in a cell size sensing mechanism.

Thus fission yeast seems to maintain coordination between growth and cell division with cell size controls over DNA synthesis and nuclear division. The latter size control could, however, also be the consequence of a constant timing period between DNA synthesis and nuclear division.

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Infective transmission and characterisation of a C-type virus released by cultured human myeloid leukaemia cells

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A C-type virus isolated from long term cultures of myeloid cells from a patient with acute myelogenous leukaemia is infectious for a wide variety of cells. The establishment of chronically infected cells enabled us to characterise the virus by biological, immunological, and biochemical tests. The virus is closely related to the simian sarcoma-associated virus isolated from a woolly monkey fibrosarcoma.

THE possibility that C-type RNA tumour viruses are involved in human neoplastic disease has been the subject of much experimentation and speculation recently. In particular, human leukaemia has come under close scrutiny because so many leukaemias from diverse species of animals such as chickens, cats, and mice, have a viral aetiology^{1,2}. Although viraemia does not accompany human leukaemia, it is possible that a C-type virus exists in a latent state and some of the well-known 'footprints' of C-type viruses have been detected in a variety of leukaemic tissues. These include: (1) cellular nucleic acid sequences homologous to viral RNA³⁻⁷; (2) presence of RNA-directed DNA polymerase (reverse transcriptase) activity akin to the viral enzyme^{3,8-14}; (3) presence of intracellular proteins that cross react in serological tests with antibodies to virus-specific proteins^{15,16}; and infrequently (4) electron microscopic identification of virus-like particles^{10,12,17,18}. In many of these reports the virus-like components in the fresh human leukaemic cells were shown to be most closely related to similar components from oncogenic primate RNA tumour viruses, especially woolly monkey (simian) sarcoma virus (SSV)^{4,5,7,14-16}.

Gallagher and Gallo¹⁹ have reported the production and preliminary characterisation of C-type virus particles from long term cultures of myeloid cells from one patient, HL23, with acute myelogenous leukaemia (AML). The uncultured blood cells of this patient contained reverse transcriptase related to that of SSV (ref. 14). The virus released by HL23 cells is called HL23V-1. In addition, they have now reported the release of similar particles from cells derived from a bone marrow specimen from the same patient obtained 14 months after the original blood specimen²⁰. This virus is called HL23V-5.

Here we show that these C-type particles are infectious. The virus may be propagated to high titre in several cell types and a quantitative bioassay is described. The production of substantial titres of virus has enabled us to characterise the virus in detail and to compare it with and show that it closely resembles the C-type SSV and its associated virus (SSAV) previously isolated from a fibrosarcoma of a woolly monkey²¹.

Infective nature of virus particles

Supernatant fluids from the AML peripheral blood cell cultures containing virus-like reverse transcriptase activity were initially inoculated on to a wide variety of animal and human cells. After transient bursts of viral replication over a period of 3 months, four chronically infected cell lines were established in A204 cells (human rhabdomyosarcoma), WHE2 cells (fibroblastic strain from a whole human embryo), A7573 (canine thymus) cells, and NRK (normal rat kidney) cells. The establishment of chronically infected virus-producing cells was therefore neither a frequent nor a rapid event. A more rapid transmission of the virus to two cell lines (A204; and KNRK, a rat kidney cell line transformed by, but not producing, Kirsten murine sarcoma virus) was obtained, however, within 3 weeks of exposure of these cells to β -propiolactone-inactivated Sendai virus immediately before inoculation with supernatant fluids from the AML cell cultures. Table 1 shows the variety of cells infected with the AML culture fluids and the frequency of infectious transmission to new host cells. The viruses produced by these new host cells are referred to as 'secondary' viruses; the nature of the host producing each secondary virus is indicated in parentheses.

The secondary HL23V-1 viruses will infect a wider variety of cells than the primary virus (Table 2). This may be a result of the virus acquiring a genuinely wider host range or more likely the result of selection by infective transmission of higher titre virus stocks and higher infectivity to particle ratios. Electron microscopy of chronically infected cultures revealed abundant C-type virus particles (Fig. 1). In contrast to the host range of SSAV, most of the HL23V-1 isolates infected 1283 marmoset cells poorly and human cells well (Tables 1–3).

Table 1 Transmission of HL23V-1 infectivity to cells of different species

Cells a, Direct infection*	Origin	1 Detection of reverse transcriptase after culture (weeks)†	2	4	7	10	12
A204	Human rhabdomyosarcoma	+	—	—	+	+	+
HT-1	Moloney MSV-transformed hamster cells	—	—	—	—	ND	ND
NC37	Human lymphoblastoid cells	—	—	—	—	—	—
WHE2	Whole human embryo	+	—	—	+	+	+
4178	Squirrel monkey lung	—	—	—	ND	ND	—
1283	Marmoset lung	—	—	—	ND	ND	—
A7573	Dog thymus	—	—	—	+	ND	+
CCL64	Mink lung	—	—	—	ND	ND	—
Tb-1-1u	Bat lung	—	—	—	ND	ND	—
BHK21	Hamster kidney	—	—	—	—	ND	—
NRK	Rat kidney	—	—	+	ND	+	+
b, Sendai-mediated infection‡		1	3	4	7		
A204	Human rhabdomyosarcoma	—	+	+	+		
CCL64	Mink lung	—	—	—	—		
KNRK	Kirsten MSV-transformed rat kidney cells	+	+	+	+		
Q226	Japanese quail embryo	—	—	—	—		

*Human acute myeloid leukaemic (AML) cells were passaged in tissue culture as previously described¹⁹. At passage 10, when the supernatant had more than a threefold higher incorporation of ³H-TMP in the presence of poly(rA)·oligo(dT) compared with poly(dA)·oligo(dT) in a reverse transcriptase reaction²⁰, subconfluent cultures of the various cell types were infected with these fluids. 10⁵ target cells were seeded in 5-ml flasks containing complete media and polybrene, 2 µg ml⁻¹. After 16 h the cells were washed and treated with 0.5 ml of cell-free supernatant from the AML cell culture (Strain I-1, ref. 20). The cultures were passaged at weekly intervals and supernatant fluids were periodically assayed for reverse transcriptase activity.

†+, ³H-TMP incorporation with poly(rA)·oligo(dT) more than twofold higher than incorporation in response to poly(dA)·oligo(dT); —, less than twofold stimulation. ND, not done.

‡The test cells were exposed to 10³ haemagglutination units of β-propiolactone-inactivated Sendai virus at 4 °C for 30 min. The cells were then washed and the AML cell culture supernatants were added in 0.2 ml volumes for 60 min at 37 °C; tissue culture medium was then added. Cell cultures were passaged at weekly intervals and assayed as mentioned above.

Quantitative plaque assay for HL23V-1

In addition to screening supernatant samples for reverse transcriptase activity, it is also possible to titrate the HL23V-1 virus isolates by an *in vitro* plaque assay test. The XC syncytial plaque assay technique²², described and developed for the murine leukaemia viruses, is also suitable for titration of the non-human primate viruses²³, simian sarcoma associated virus (SSAV) and gibbon ape leukaemia virus (GALV)²⁴. Utilising the standard technique²², we have been able to make quantitative determinations of the HL23V-1 virus isolates on various cell types (Table 3). Although the human and rhesus monkey cells show the greatest susceptibility to infection by these viruses, a linear dose-response curve is obtained on all cell lines tested.

The availability of a plaque assay affords the opportunity to study the quantitative neutralisation patterns with sera from normal human subjects, leukaemic patients, and antisera prepared specifically against animal C-type viruses such as SSV. Recently Snyder *et al.*²⁵ have shown by radioimmunoassays that all human sera contain antibodies to the major proteins and glycoproteins of several C-type viruses, including SSV.

We are currently screening a variety of sera, by plaque neutralisation tests and by a radioimmunoprecipitation assay, for antibodies that cross react with the HL23V-1 isolates. Our tests so far indicate that antibodies that react with HL23V-1 are widely represented in the human population (R. Kurth, T. Oliver, R. W. and N. M. T., unpublished).

Formation and envelope specificity of murine sarcoma virus pseudotypes rescued by HL23V-1

Murine sarcoma virus (MSV) strains are defective for replication and require leukaemia viruses for propagation of infectious stocks. The leukaemia 'helper' virus provides envelope antigens, reverse transcriptase and perhaps other viral structural components for the MSV and the complemented, infectious MSV particles are called pseudotypes. 'Non-producer' cells transformed by MSV can be obtained by infection with high dilutions of MSV, and infectious MSV pseudotypes can be rescued on superinfection of the cells with helper virus. Leukaemia viruses of other species, such as feline leukaemia virus, GALV and SSAV will also rescue MSV pseudotypes on superinfection of susceptible non-producer cells. The KNRK cells

Table 2 Infectivity of secondarily-passaged HL23V-1 viruses as measured by reverse transcriptase activity

Cells	Origin	c.p.m. ³ H-TMP incorporated per ml per 30 min (× 10 ⁻⁴) after infection with			
		HL23V-1 (A204)	HL23V-1 (KNRK)	HL23V-1 (A7573)	HL23V-1 (WHE2)
A204	Human rhabdomyosarcoma	207	114	188	0.4
HRU-1	Human uterus	96	152	132	1.0
NC37	Human lymphoblasts	18	45	ND	ND
WHE1	Whole human embryo	68	282	168	0.7
WHE3	Whole human embryo	10	80	ND	ND
WI38	Human lung	157	185	ND	ND
CCL64	Mink lung	35	91	48	0.2
Tb-1-1u	Bat lung	397	539	410	0.1
SIRC	Rabbit cornea	198	404	162	12
1283	Marmoset lung	2.3	80	2.6	0.4
NRK	Normal rat kidney	78	53	85	1.1
KNRK	KiMSV-transformed NRK	640	757	71.1	91

Cells of each cell type were seeded at 3.5 × 10⁵ cells per flask (Falcon T30) and infected on the following day in medium containing 20 µg polybrene ml⁻¹. Medium was changed every 2 d and the fluids were collected on day 8 for assay of reverse transcriptase activity. Corrections for background c.p.m. have been subtracted.

used for the propagation of HL23V-1 are transformed, non-producer cells containing the genome of Kirsten-MSV. We therefore assayed the HL23V-1(KNRK) virus for focus-forming units (FFU) on NRK, 7605L, mink, and Japanese quail cells and found titres of up to 10^6 FFU ml⁻¹ on all these cell types. The MSV(HL23V-1) pseudotype from infected KNRK cells plated with a linear dose response on NRK cells. The other HL23V-1 isolates, which were not propagated in MSV-containing cells, did not contain focus-forming virus in our assay, but could act as helper viruses. For example, when MSV-containing human cells (kindly provided by Dr P. Peebles) were super-infected with HL23V-1 (A7573), MSV pseudotypes were rescued at a titre of 2×10^4 FFU ml⁻¹.

The MSV pseudotype focus assay can be used as an alternative to the XC syncytial plaque assay in neutralisation tests with antisera. A 1% dilution of antiserum to SSV raised in a goat (kindly provided by Dr F. Deinhardt) which gave a 1,000-fold reduction in titre of MSV(SSAV) neutralised MSV(HL23V-1) to a similar degree. Serum from patient HL23, from whom the virus-producing myeloid cell line was originally derived, did not significantly neutralise MSV(HL23V-1) or MSV(SSAV) pseudotypes. Preliminary tests, however, indicate that weak neutralising activity (approximately 10-fold reduction of FFU using 10^{-1} dilutions of serum) is evident in the sera of some normal individuals and patients in remission stages of myelogenous leukaemia.

The MSV pseudotypes were also used in a virus interference test. 7605L foetal human lung fibroblasts cells were infected with HL23V-1(A7573) or GALV and were challenged after three passages with MSV(HL23V-1) and MSV(SSAV) propagated in KNRK cells. Both leukaemia viruses reduced the plating efficiency of the two MSV pseudotypes to less than 1% that on uninfected 7605L cells. This indicates relatedness among cell receptor sites for SSAV, GALV and HL23V-1. The combined results from neutralisation and interference assays suggest that the envelope proteins of the three groups of viruses are genetically related.

Immunological characterisation of proteins of the HL23V-1 isolates

Biochemical and immunological characterisation of the HL23V-1 virus strains was carried out to evaluate the relationship of the secondary virus isolates to (1) the parental virus isolate, (2) the other secondary virus isolates, and (3) the non-human primate viruses, SSV and GALV.

Antibodies to the reverse transcriptase enzymes from the different C-type viruses have become valuable tools for studying immunological relatedness among these viruses. A quantitative assay for neutralisation of reverse transcriptase activity used antisera prepared specifically against the purified viral enzymes^{11,13,19}. We used this technique to analyse the serological specificity of the enzyme activity in supernatant fluids from the myeloid cells producing the primary virus and the cells producing the secondary virus isolates (Table 4).

The viruses produced by the AML cells in culture, HL23V-1

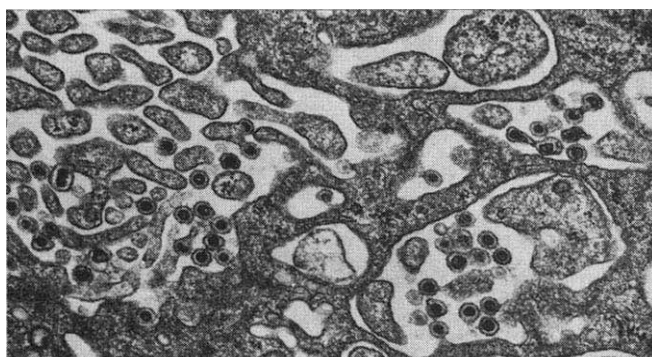


Fig. 1 C-type particles in HL23V-1-infected KNRK cells ($\times 18,600$).

(ref. 19) and HL23V-5 (ref. 20) from peripheral blood and bone marrow, respectively, obtained from the patient several months apart, show nearly identical patterns in these assays of inhibition of reverse transcriptase activity. They are most strongly inhibited by antiserum prepared against the SSV enzyme, less strongly inhibited by anti-GALV enzyme and inhibited least by antiserum to RD114 enzyme (Table 4). This last neutralisation pattern is also seen when purified SSV and GALV enzymes are treated with the RD114 antiserum. By these criteria, the HL23V isolates are most closely related to SSV and GALV.

The specificity of the reverse transcriptases of the secondarily propagated viruses gave much the same pattern as the primary isolates. One interesting anomaly is the HL23V-1 virus grown in A204 cells which may contain two C-type viruses. As with the protein analysis (see below) and the low XC plaque titres, this virus isolate shows a larger cross reaction to RD114 than the other isolates. Also noteworthy is the shift in this pattern with continued passage (compare experiments 1 and 2) which could represent the selection of the SSV-like component over the RD114-like component. Further studies are in progress to elucidate the basis of this phenomenon.

Other tests (not shown) using antisera prepared against the enzymes of feline leukaemia virus, Rauscher murine leukaemia virus, Mason-Pfizer virus, avian myeloblastosis virus, and human cellular polymerase α show little or no inhibition of the HL23V-1 viral reverse transcriptase.

The secondary cells infected by HL23V-1 contained proteins that bound antibodies prepared against p30 protein²⁶ of SSV and GALV but not with antisera prepared against p30 of mouse or rat leukaemia viruses (R. Gilden, personal communication). In addition, secondarily-infected A204 and A7573 cells contained a protein that reacted with antisera prepared against p30 of baboon endogenous virus and, to a lesser extent, RD114. The p30 proteins of GALV and SSV are not distinguishable by immunological cross-reaction assays²⁷; therefore the relatedness of p30 from HL23V-1 to p30 of SSV and GALV indicates that

Table 3 XC syncytial plaque assay of HL23V-1 isolates

Cells	Plaque-forming units per ml of virus				SSAV
	HL23V-1 (A204)	HL23V-1 (KNRK)	HL23V-1 (A7573)	HL23V-1 (WHE2)	
7605L	2.9×10^4	4.8×10^7	7.5×10^5	5.0×10^3	5.0×10^3
WHE2	1.9×10^5	3.0×10^6	5.8×10^5	1.9×10^3	3.0×10^3
FRhL-1	3.6×10^3	2.3×10^6	1.1×10^5	1.0×10^3	3.7×10^5
1283	5.0×10^1	5.5×10^4	5.0×10^2	1.0×10^2	2.0×10^4
NRK	1.5×10^2	7.7×10^5	1.7×10^5	$< 5.0 \times 10^3$	3.5×10^5
SIRC	$< 5.3 \times 10^3$	7.5×10^4	5.5×10^4	$< 5.0 \times 10^3$	5.0×10^3

Cells were seeded at 2×10^5 per 35-mm diameter plates (Linbro) and infected on the following day with 0.1-ml volumes of serially 10-fold diluted virus stocks in the presence of $20 \mu\text{g ml}^{-1}$ polybrene. Assays were done according to the standard XC plaque method. Reverse transcriptase assays on the various input virus inocula were determined; ³H-TMP incorporated per ml of undiluted virus in a 30 min reaction with poly(rA)-oligo(dT): 6.9×10^6 c.p.m. for HL23V-1 (A204); 1.2×10^7 c.p.m. for HL23V-1 (KNRK); 2.5×10^6 c.p.m. for HL23V-1 (A7573); and 5.8×10^5 c.p.m. for HL23V-1 (WHE2). Cells not mentioned previously: 7605L, foetal human lung fibroblasts (kindly provided by Dr E. H. Macintyre) and FRhL-1, foetal rhesus monkey lung cells (US Division of Biological Standards).

Table 4 Inhibition of reverse transcriptase by antisera to RD114, GALV and SSV

Source of reverse transcriptase	% Inhibition of reverse transcriptase activity by					
	anti-RD114*		anti-GALV†		anti-SSV‡	
	Expt 1§	Expt 2§	Expt 1§	Expt 2§	Expt 1§	Expt 2§
Primary virus-producing cells‡						
HL23V-1	25	33	65	58	78	68
HL23V-5	25	27	47	51	88	52
Secondary virus-producing cells						
HL23V-1 (A204)	60	37	23	54	N.D.	61
HL23V-1 (KNRK)	38	36	64	74	N.D.	83
HL23V-1 (A7573)	40	36	70	53	N.D.	54
HL23V-1 (WHE2)	33	19	68	67	N.D.	77
Immunising enzymes						
RD114	60–70¶		10–25¶		15–25¶	
GALV	10–20		80–90		80–90	
SSV	20–30		80–95		80–95	

Antisera reactions and reagents are described elsewhere¹⁹. Inhibition represents the reduction in DNA polymerase activity, using the primer-template poly(rA):oligo(dT) and ³H-TTP, with an immune serum compared to that with non-immune serum. DNA polymerase activity did not significantly differ when non-immune serum was replaced by 0.1 M Tris, pH 8.0. Values represent the average of duplicate determinations in assays with a minimum of 15,000 c.p.m. above background in the absence of immune serum.

*Antiserum to reverse transcriptase of RD114, an endogenous virus of domestic cats^{28–31}, which also selectively inhibits the reverse transcriptase of an endogenous primate virus isolated from normal baboon tissues^{32,33}. Antiserum kindly provided by Dr R. Gilden.

†Antisera to reverse transcriptases of the two primate C-type viruses. Preparation of antisera in rabbits and rats has been described previously¹⁹.

‡HL23V-1, C-type virus produced by cultured myelogenous leukaemia cells from peripheral blood of patient HL23. HL23V-5, C-type virus from cultured myelogenous leukaemia cells from a bone marrow specimen of the same patient.

§Mean values from determinations achieved with different collections of media in January 1975 (expt 1) and in March 1975 (expt 2) after further passage of the same cultures.

¶Ranges from multiple assays.

all three viruses are members of the same family, not that they are identical.

In a competitive radioimmune assay for p12 viral protein, secondary cells infected by HL23V-1 contain a protein that is indistinguishable from p12 protein of SSV but unrelated to p12 from GALV (Tronick, Stephenson, and Aaronson, personal communication).

Nucleic acid hybridisation

For molecular hybridisation studies using viral RNA, the supernatant fluids of infected cells were clarified, centrifuged through sucrose gradients, and the poly(A)-containing 70S RNA was extracted. All of the HL23V-1 secondary viruses had characteristic 70S RNA and these purified RNAs were then subsequently used for hybridisation reactions.

The viral RNAs were hybridised with cellular DNA of uninfected NRK or KNRK cells or SSV-infected KNRK cells to analyse the genetic relatedness of the HL23V-1 isolates to SSV (Table 5). All the HL23V-1 RNAs show more hybridisation to DNA from those cells infected by SSV than to DNA from uninfected cells. Therefore a qualitative relationship exists

between the nucleic acid of SSV and those of the HL23V-1 isolates. Further studies to determine the degree of homology between the different viral RNAs, by hybridisation between the viral RNAs and their complementary DNA transcripts, are in progress. It is interesting to note the low degree of hybridisation seen between the viral RNAs and DNA from normal human cells; this would indicate that viral genetic material related to HL23V-1 is not maintained as an endogenous genetic component in humans, at least not in all cells.

To elucidate further the genetic relatedness between HL23V-1 and SSV, hybrids formed between SSV 70S RNA and infected and uninfected cellular DNA were subjected to an analysis of thermal stability (Fig. 2). From such profiles, the *t_m* values (the temperature at which 50% of the hybrid is dissociated) indicate the extent to which the hybrids are well matched in base pairing. The *t_m* curves are rather broad, indicating heterogeneity in provirus DNA and/or in virus RNA. As can be expected, however, the hybrids formed between SSV RNA and DNA from cells from two different species infected with SSV have a high *t_m* and therefore better matching of base pair sequences than do the hybrids with normal gibbon ape DNA, cells infected with GALV, or cells infected with one of the HL23V-1 viruses.

Table 5 Genetic relationship between HL23V-1 RNA and SSV provirus DNA

Cell DNA	% Hybridisation of viral RNAs					
	HL23V-1 (A204)	HL23V-1 (KNRK)	HL23V-1 (A7573)	HL23V-1 (WHE)	SSV (NRK)*	SSV (71AP1)*
NRK	4	25	16	5	20	26
KNRK	7	28	15	13	17	34
KNRK (SSV)†	33	65	48	57	49	49
Normal human	7	3	10	3	5	3
None	2	1	2	0	4	2

Virus-producing cells were labelled for 48 h with 100 μ Ci ml⁻¹ ³H-uridine. Media was freed of cells by centrifugation at 1,000g for 10 min, adjusted to a final concentration of 1% SDS, 0.1% polyvinylsulphate, and 0.3 M NaCl, and passed over an 0.2 ml bed volume column of oligo-(dT)-cellulose at 25 °C and 1 ml min⁻¹. Poly(A)-containing RNA was eluted with 10 mM Tris, pH 7.4 and centrifuged for 2 h at 22 °C in 5–20% sucrose gradients containing 0.5% SDS, 0.01 M Tris, pH 7.4, 0.1 M NaCl and 0.001 M EDTA. The 70S RNA was used in hybridisation experiments. Hybridisation mixtures of 0.1 ml consisted of 300 c.p.m. of ³H-RNA (0.3–1 ng), 1 mg of DNA, and 0.3 M PO₄ pH 6.8. The mixtures were flame-sealed in capillary tubes, boiled for 5 min, and then incubated at 60 °C for 7 d (*C₀t* = 20,000 uncorrected for ionic strength). The contents of the capillary tubes were expelled into 0.8 ml of 2×SSC (0.3 M NaCl, 0.03 M Na citrate, pH 7.0). A 0.2 ml aliquot was precipitated with 10% trichloroacetic acid (TCA); the remaining 0.6 ml aliquot was incubated with 20 μ g ml⁻¹ of RNase for 2 h at 37 °C, then precipitated with TCA. The precipitates were collected on nitrocellulose membrane filters, incubated for 1 h at 60 °C with 1 ml of 0.2 M NaOH mixed with 1 ml 50% acetic acid, and counted in 10 ml of Aquasol after incubating 24–36 h at 5 °C in the dark. No TCA-precipitable radioactivity was lost during hybridisation at 60 °C. Percentage RNA hybridised = (c.p.m. in RNase-treated aliquot) (c.p.m. in 0.2 ml aliquot × 3)⁻¹ × 100.

*SSV grown in NRK rat cells or in 71AP1 marmoset cells.

†KNRK cells infected with SSV.

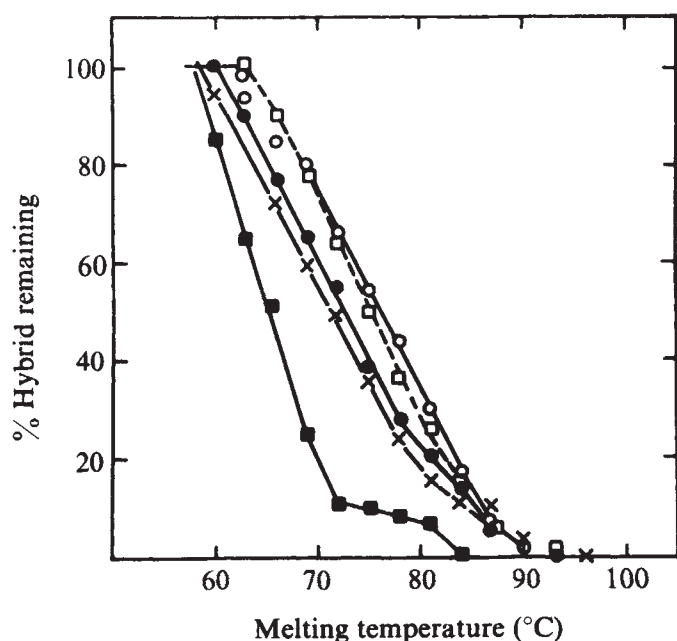


Fig. 2 Thermal stability profiles of hybrids formed between SSV 70S RNA and infected cellular DNAs. 32 P-70S RNA isolated from simian sarcoma virus (SSV) produced by 71A1 marmoset cells ($15,000$ c.p.m.; 5×10^7 c.p.m. μg^{-1}) was hybridised at 60°C in 0.4 M PO_4 to $1,000$ μg of DNA isolated from the indicated cells. Hybridisation was carried out to a C_0t of $20,000$ with respect to DNA concentration and uncorrected for (Na^+) . Hybrid solutions were expelled into a 100 -fold volume excess of 0.15 M Na^+ , divided into 0.5 ml aliquots, and held at the indicated temperature for 5 min. The solution was brought to final concentrations of 0.45 M Na^+ and 20 μg ml^{-1} RNase, and the sample was incubated for 2 h at 37°C . Acid-insoluble material was collected on nitrocellulose membrane filters. ○, HF marmoset cells infected by and producing SSV; □, rat cells infected by and producing SSV; ●, human rhabdomyosarcoma cells infected by and producing HL23V-1; ×, human lymphoblastoid cells infected by and producing gibbon ape leukaemia virus; ■, fresh brain tissue from an apparently normal gibbon ape. The percentage SSV RNA hybridised was 9% with normal gibbon DNA and 25 – 50% with DNA from virus-infected cells.

Although the hybridisation and immunological assays of genetic similarity show that the nucleic acids and proteins of HL23V-1 and SSV are closely related, the t_m experiment suggests that genomic differences do exist between HL23V-1(A204) and SSV. More complete analysis will be presented elsewhere.

Possible implications

It is not surprising to find a C-type virus associated with acute myelogenous leukaemia; viral-related proteins and nucleic acid sequences have been detected in a variety of leukaemias and sarcomas in humans. We have shown here, however, that such virus-related markers can be associated with an infectious virus particle. Freshly isolated leukaemia cells do not release readily detectable C-type virus and the capacity to maintain and propagate the myeloid leukaemic cells in culture for extended periods of time may have allowed production of transmissible virus in this case. Myeloid cell cultures established from an independent, later biopsy of the same patient also release virus particles as measured by reverse transcriptase activity²⁰. We have found that this virus, designated HL23V-5, is also infectious for human cells and can be titrated by the XC syncytial plaque assay.

The clear resemblance of the HL23V-1 viruses from human AML cells to the SSAV isolated from a fibrosarcoma of a woolly monkey is striking. The viruses are markedly similar by biological, immunological and molecular criteria. But our data on the relative plating efficiencies on human and 1283 marmoset cells, the inhibition of reverse transcriptase activity

by specific antisera, and the nucleic acid hybridisation studies suggest that, though closely related, the HL23V-1 virus and SSV are not identical. Many precautions were taken to ensure against the possibility of a laboratory contamination¹⁹; there was no SSAV grown or handled in the biohazard facility in which the myeloid cells or chronically infected cells were maintained. Should we then be surprised by the relatedness of the simian and human viruses? One interesting fact is that the SSV(SSAV) viruses were obtained from a woolly monkey that had been a pet¹⁴ and was therefore in close contact with a human population. We do not know of any C-type viruses isolated from other woolly monkeys. If the HL23V-1 virus and SSAV do indeed have a common origin, it is plausible that the monkey was infected by the human virus. Preliminary serological studies indicate that antibodies to the virus are widespread in the human population. Moreover, a closely related virus has been isolated several times from normal human embryo cells by Panem *et al.*³⁴, and Nooter *et al.* describe elsewhere in this issue the isolation of an SSV-related virus from human leukaemic cells³⁵. We should bear in mind, however that isolation of the virus from leukaemic cells does not necessarily mean that the virus is aetiologically related to the leukaemia. Tests are currently in progress to determine whether the virus has oncogenic potential in animals.

Our characterisation of the infectious nature of this virus may be useful in detection and propagation of similar viruses from freshly-isolated leukaemic and solid tumour tissues.

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Multiple steps in DNA recognition by restriction endonuclease from *E. coli* K

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The process of DNA recognition by the activated form of the restriction endonuclease from E. coli K involves three enzyme-DNA complexes which can be differentiated experimentally. These are: an initial complex formed at a nonspecific site; a recognition complex involving the host specificity site; and a cleavage complex dependent on the presence of ATP.

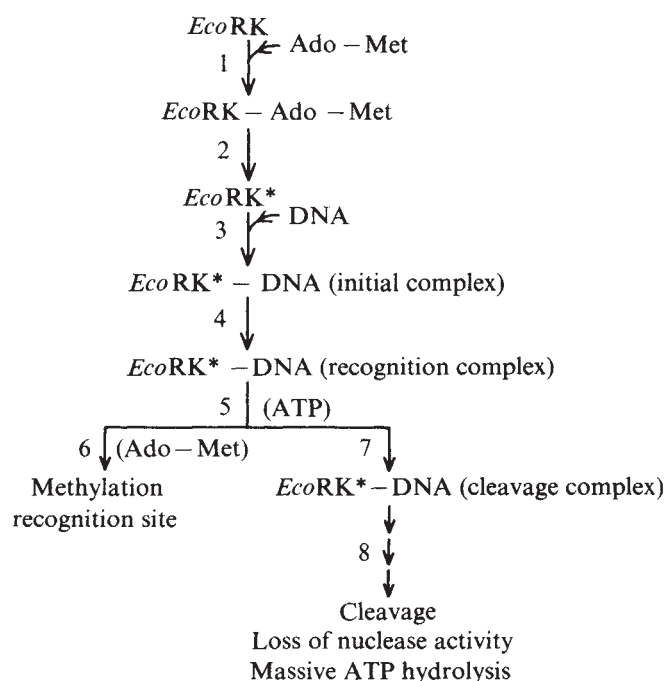
THE restriction endonuclease from *Escherichia coli* K, *Eco*RK, is a complex multifunctional protein which cleaves unmodified DNA in the presence of S-adenosylmethionine (Ado-Met), ATP and Mg^{2+} (ref. 1). A powerful ATPase activity appears during the course of this restriction reaction² and the same protein is also able to methylate unmodified DNA, rendering it insensitive to its own restriction activity³.

The way in which the enzyme recognises the host specificity sequence on the DNA, and the factors that determine whether it will act as an endonuclease, or as a methylase, are two aspects of the mechanism which go beyond the immediate area of restriction and modification. The first of these, recognition of nucleotide sequences, is common to most proteins that react with nucleic acids. The second is that the interaction involved in the recognition of the nucleotide sequence directs the protein towards either of two opposed modes of action, restriction or modification. In other words, a specific nucleotide sequence may not only be recognised by a protein, but also act as a signal for the mode of action of a multifunctional enzyme.

In a certain perverse way, it is the very complexity of this protein and the reactions that it carries out that enables us to distinguish experimentally four major stages in the *in vitro* reaction (Fig. 1). These are: Enzyme activation: *Eco*RK binds Ado-Met rapidly, and in a slow second step, an allosteric transition to an activated form (*Eco*RK*) takes place. DNA recognition: *Eco*RK* interacts with DNA, first, at a nonspecific site (initial complex), and then at the unmodified recognition site (recognition complex). The recognition site is the host specificity site on the DNA that confers susceptibility to a given restriction and modification system (the sites for the K system are called sK). Enzyme action: in the absence of methyl groups at the recognition site, the DNA will be either cleaved or methylated. Methylation occurs at the recognition site while DNA scission takes place elsewhere. Loss of nuclease activity: the enzyme is altered following DNA cleavage, and is unable to cleave again. This phenomenon is probably related to the appearance of a vigorous and sustained ATP hydrolysis which begins at the time of DNA cleavage, but continues long after the DNA digest has reached its limit.

This paper deals with the process of DNA recognition. *Eco*RK* can form three distinct complexes with DNA in its restriction mode. It first forms an initial complex at a nonspecific site which is transformed to a more stable recognition complex if an unmodified host specificity site is present on the DNA. Neither of these complexes can be trapped on membrane filters. If ATP is added to the recognition complex, a rapid transition to a third complex which can be trapped on filters, takes place. This presumably occurs at the cleavage site.

Fig. 1 Reaction mechanism of endonuclease *Eco*RK.



Enzyme forms a specific complex with unmodified DNA that is retained on filters

*Eco*RK has previously been shown to form a specific complex with unmodified λ .0 DNA that can be stabilised with EDTA and detected by binding to nitrocellulose filters. (λ bacteriophage grown on a non-modifying host is designated λ .0; phage grown on a host that confers K-specific modification to the DNA is called λ .K.) The formation of this complex requires the presence of Ado-Met, ATP and Mg^{2+} (ref. 4). No filter-binding complex can be detected with modified λ .K DNA or with DNA that contains no recognition sites for the enzyme. Complex formation reaches its maximum after 2 min at 30 °C, and then decays at a rate consistent with its dissociation after DNA cleavage.

That this complex was not the result of binding to sK sites on the DNA was suggested by experiments with a purified restriction endonuclease from a mutant strain of *E. coli* K12 (ref. 5). This strain is unable to restrict, but can modify normally. As expected, the restriction enzyme purified from it can methylate (but cannot cleave) unmodified DNA *in vitro*. It does not form the filter binding complex. Therefore, *Eco*RK can recognise and methylate λ .0 DNA without forming a filter binding complex. The conclusion then is that the filter-binding complex must be associated with the cleavage reaction, and not DNA recognition proper.

Activated enzyme is stabilised by interaction with DNA

*EcoRK** forms two different types of complex with DNA: a nonspecific initial complex and a specific recognition complex. These two complexes can be readily differentiated by observing the effect of λ .K, λ .0 and mutant λ DNAs on the stability of *EcoRK**.

The slow allosteric activation to *EcoRK** follows the rapid binding of Ado-Met to *EcoRK*⁶. On dilution to remove free Ado-Met, *EcoRK** decays with a half life of 130 s. The effect of DNA on the stability of *EcoRK** was determined by adding modified λ .K or λ sK₁⁰sK₂⁰.0 DNAs to the dilution buffer. The latter DNA is from a λ - Φ 80 hybrid in which all of the sK sites have been eliminated⁷. Clearly both λ .K and λ sK₁⁰sK₂⁰.0 DNAs stabilise *EcoRK** (Fig. 2a) and thus interact with it, although neither of them is a substrate for the enzyme. Similar stabilisation was observed with λ .0 and T4 DNAs. We define this kind of interaction as initial complex formation.

To detect differences in the interaction between *EcoRK** and the various DNAs, the enzyme was activated in the presence of λ .0, λ .K or λ sK₁⁰sK₂⁰.0 DNAs and the stability of the complexes formed in these conditions was measured over a period of 60 min following dilution into prewarmed buffer (compared with 6 min in the previous experiment). Figure 2b shows that, although all of the DNAs protect *EcoRK** from decay, substrate λ .0 DNA is more efficient than the others. The half life of the activated enzyme goes from 130 s for the enzyme alone to 6 min in the presence of the non-substrate λ .K and λ sK₁⁰sK₂⁰.0 DNAs and 22.5 min with substrate λ .0 DNA. This enables us to postulate an initial complex formed by *EcoRK** with any DNA, and a more stable recognition complex formed with a DNA that contains the unmodified sK sequence.

Recognition complex is a precursor of the filter-binding complex

The complex formed by activating the enzyme in the presence of λ .0 DNA (that is, the recognition complex) does not bind to nitrocellulose filters, but the addition of ATP converts it to the filter-binding form. The rate at which this occurs seems to follow first-order kinetics with a half time for the reaction of 6.5 s. This is more than 10 times faster than the result obtained when *EcoRK** is added to λ .0 ³²P-DNA and ATP (Fig. 3a), also a first-order reaction with a half time of about 80 s. Given these rapid binding kinetics, it cannot be ruled out that the recognition complex is, in fact, formed at the cleavage site after interaction with the sK site. Also in this figure are the kinetics of filter binding complex formation when a standard assay was initiated by the addition of non-activated enzyme, and when *EcoRK** was diluted into λ .0 ³²P-DNA, and ATP was added 2 min later. In the latter case, no DNA binding was observed. Therefore, the rate at which the recognition complex was formed could not be measured directly.

The rate of activation of the enzyme in the presence of unmodified DNA is first or pseudo-first order (Fig. 3b) and is faster than activation of the enzyme alone⁶ (respective half lives of 36 s as against 54 s). Therefore, interaction of the enzyme with DNA displaces the equilibrium towards complex formation and prevents the decay of *EcoRK**.

The reaction of the recognition complex with ATP should be second order; therefore some subsequent rate-limiting change in the structure of the enzyme-DNA complex which generates the filter-binding form may explain the apparent first-order kinetics. The recognition complex formed in the absence of ATP was shown to be an intermediate in the restriction pathway. On dilution into ATP, the DNA was rapidly cleaved. The initial complex formed between *EcoRK** and λ .K DNA (on dilution into λ .0 ³²P-DNA and ATP) produced filter-binding kinetics that were faster than the standard reaction rate and similar to

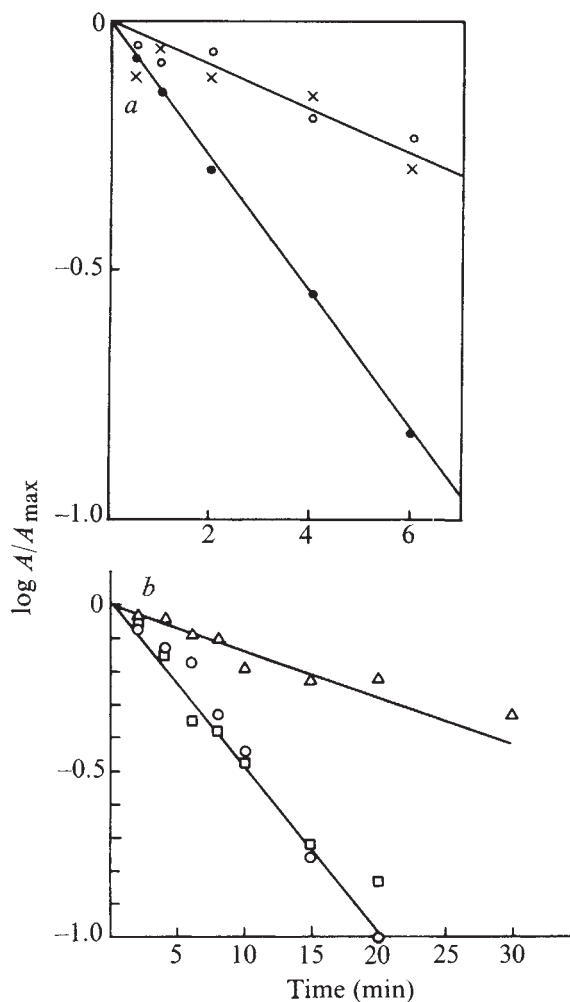


Fig. 2 Effect of DNA on the stability of *EcoRK**. *a*, Stability of *EcoRK** in presence or absence of DNA. *b*, Comparison of effect of different DNAs. *a*, λ .K and λ .0 ³²P-DNA were prepared as described previously¹. λ sK₁⁰sK₂⁰.0 DNA was prepared from phage after heat induction of 803 ($h^{80}att\lambda sK_2^0 cI_{857} sK_1^0$), obtained from Dr N. Murray. *EcoRK* was purified to homogeneity after modifying the procedure published elsewhere¹. All reaction mixtures for this and subsequent experiments contained 6 mM MgCl₂, 0.26 mM EDTA, 12 mM 2-mercaptoethanol and 100 mM either Tris-HCl or HEPES-OH, pH 8.0. Other additions are detailed in Figure legends. An aliquot (70 μ l) of reaction mixture containing 0.13 nmol Ado-Met was mixed with 50 μ l (50 μ g) of *EcoRK*, incubated for 5 min at 30 °C, chilled and kept on ice. Aliquots (3 μ l) were then diluted into 300 μ l prewarmed reaction buffer containing either no DNA, 0.3 μ g of λ .K DNA, or 0.3 μ g λ sK₁⁰sK₂⁰.0 DNA. After incubation for varying periods, 5 μ l of the mixture containing 80 nmol ATP and 0.1 μ g λ .0 ³²P-DNA (16,700 c.p.m.) were added and the incubation continued for a further 2 min. The reaction was terminated by the addition of 50 μ l 0.5 M EDTA and filtered through a nitrocellulose filter. The filters were washed, dried and counted as described previously¹. *b*, Three reaction mixtures of 100 μ l containing 0.13 nmol Ado-Met each and either 2.4 μ g λ .0 ³²P-DNA (380,000 c.p.m.), 2.2 μ g λ .K DNA or 2.4 μ g λ sK₁⁰sK₂⁰.0 DNA were prepared. They were incubated at 30 °C for 2 min with 30 μ l (6 μ g) *EcoRK* and then rapidly diluted with 11.5 ml prewarmed reaction buffer. Aliquots (500 μ l) were removed at various intervals and mixed with either 20 μ l ATP mixture (incubation containing λ .0 ³²P-DNA) or 20 μ l ATP-DNA mixture (the other two incubations). ATP mixture contained 0.2 nmol ATP per 20 μ l and ATP-DNA contained 0.2 nmol ATP and 96 ng λ .0 ³²P-DNA per 20 μ l (15,200 c.p.m.). After addition of the ATP mixture, further incubation was for 1 min, whereas after adding the DNA-ATP mixture the incubation was for 2 min. All reactions were terminated by the addition of 0.5 ml 0.1 M EDTA, filtered and counted as above. ●, No DNA; Δ, λ .0 DNA; ×, λ .K DNA; ○, λ sK₁⁰sK₂⁰.0 DNA.

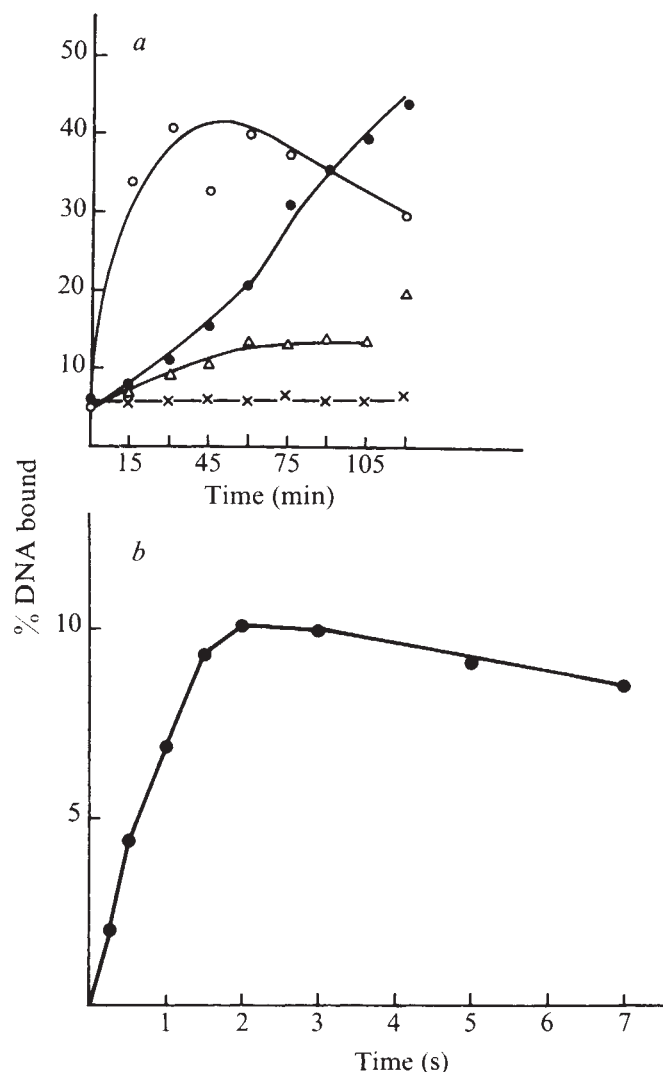


Fig. 3 Kinetics of enzyme-DNA interactions. *a*, Kinetics of DNA binding to filters after ATP addition. *b*, Kinetics of enzyme activation in presence of DNA. *a*, Standard reaction kinetics were followed by adding 9 μ l *EcoRK* (1.8 μ g) to a prewarmed reaction mixture containing in 4.5 ml: 1.6 μ g λ .0 32 P-DNA (2.16×10^5 c.p.m.), 1.8 μ mol ATP and 120 nmol Ado-Met. Aliquots (250 μ l) were removed into 25 μ l 0.5 M EDTA after different times of incubation at 30 $^{\circ}$ C and the samples were then assayed on filters. *EcoRK** was formed by adding 18 μ l *EcoRK* (3.6 μ g) to 190 μ l reaction mixture containing 1 nmol Ado-Met and incubating for 3 min at 30 $^{\circ}$ C. This was chilled in ice and 5 μ l aliquots were added to 250 μ l reaction mixture containing 0.09 μ g λ .0 32 P-DNA (1.2×10^4 c.p.m.) and 0.1 μ mol ATP. Incubation was carried out for varying time intervals, the reaction was stopped with EDTA and the samples were filtered. In a separate set of incubations, 5 μ l of the same *EcoRK** was added to 250 μ l of a similar mixture, but without ATP, the incubation continued for 3 min and 0.1 μ mol ATP was then added. After further incubation at 30 $^{\circ}$ C for varying time intervals, the reactions were terminated with EDTA and filtered. In the last set of reactions, the *EcoRK**- λ .0 DNA recognition complex was formed by adding 9 μ l (1.8 μ g) *EcoRK* to 90 μ l reaction mixture containing 2.4 nmol Ado-Met and 1.6 μ g λ .0 32 P-DNA (2.16×10^5 c.p.m.) and incubating for 3 min at 30 $^{\circ}$ C. The mixture was chilled in ice and 5 μ l aliquots were added to 250 μ l reaction mixture containing 0.1 μ mol ATP. The samples were incubated for different time intervals at 30 $^{\circ}$ C, stopped with EDTA and filtered. *b*, Reaction mixture (60 μ l) containing 2.75 μ g λ .0 32 P-DNA (2.2×10^5 c.p.m.), 0.13 nmol Ado-Met and 1 μ l (0.2 μ g) *EcoRK* was incubated at 30 $^{\circ}$ C, and at different times 3 μ l aliquots were removed and mixed with 200 μ l prewarmed buffer containing 0.1 μ mol ATP. Each sample was then incubated for a further 2 min, 50 μ l 0.5 M EDTA was added and the samples were filtered and counted as before. $\bullet$, Standard reaction kinetics; Δ , kinetics with *EcoRK**; $\times$, incubation of *EcoRK** with DNA in absence of ATP; $\circ$, kinetics of filter binding with preformed recognition complex.

the rate when *EcoRK** is added to λ .0 32 P-DNA and ATP. Dissociation of the enzyme from such initial complexes must therefore be a relatively rapid process and the activated enzyme must interact with many different DNA molecules during protection by non-substrate DNA (compare the half life for protection of 6.5 min with the rapid release detected above).

Recognition complex is insensitive to heparin

The polyanionic glycan, heparin, is an inhibitor of both the DNA binding and cleavage reactions (R. Y., T. A. B., W. E., and C. B., unpublished). This inhibition has been used to study the interactions between the enzyme and substrate DNA. Table 1 shows the results of one such series of experiments. The addition of

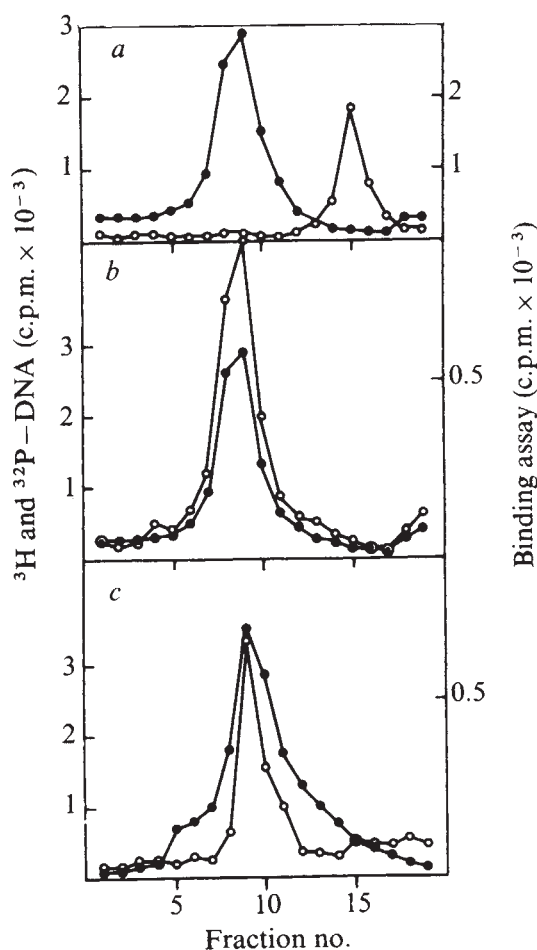


Fig. 4 *EcoRK**-DNA complexes on glycerol gradients. *a*, Composite showing the positions of *EcoRK** and λ DNA. *b*, *EcoRK**- λ .0 DNA complex. *c*, *EcoRK**- λ .K DNA complex. Four reaction mixtures of 100 μ l containing: *a*, 0.45 μ g λ .0 32 P-DNA (63,000 c.p.m.); *a*, 6.7 nmol Ado-Met and 5 μ l (1 μ g) *EcoRK*; *b*, 0.45 μ g λ .0 32 P-DNA (63,000 c.p.m.), 6.7 nmol Ado-Met and 5 μ l (1 μ g) *EcoRK*; *c*, 0.57 μ g λ .K 32 H-DNA (132,000 c.p.m.), 6.7 nmol Ado-Met and 5 μ l (1 μ g) *EcoRK*. All reactions were incubated at 30 $^{\circ}$ C for 3 min, chilled in ice and layered on 4 ml 10-30 % glycerol gradients containing 20 mM potassium phosphate, pH 7.0, 5 mM 2-mercaptoethanol and 0.2 mM EDTA. These were centrifuged at 55,000 r.p.m. for 100 min at 4 $^{\circ}$ C in an international SB 405 rotor. Twenty fractions were collected from each gradient and 50 μ l aliquots were removed from each fraction from *a*, *b* and *c* for counting. Aliquots (50 μ l) of *a*' and *c* were assayed by the standard procedure except that Ado-Met was omitted from the incubation. Aliquots from *b* were assayed similarly except that both Ado-Met and additional λ .0 32 P-DNA were omitted.

Table 1 Inhibition by heparin of DNA binding to filters by *EcoRK*

First incubation	Second incubation	λ .0 32 P-DNA bound (c.p.m.)	% Maximum
Set 1			
1 Complete	—	6,565	100
2 Complete+heparin	—	5,569	84.8
		80	1.2
3 Complete—enzyme	—	70	1.1
		46	0.7
		55	0.8
Set 2			
4 <i>EcoRK</i> +Ado-Met	λ .0 DNA+ATP	2,185	86
		2,541	100
5 <i>EcoRK</i> +Ado-Met	λ .0 DNA+ATP	46	1.8
+heparin		35	1.4
6 <i>EcoRK</i> +Ado-Met	λ .0 DNA+ATP+heparin	35	1.4
		77	3.0
Set 3			
7 <i>EcoRK</i> +Ado-Met+ λ .0 DNA	ATP	1,682	87.9
		1,913	100
8 <i>EcoRK</i> +Ado-Met+ λ .0 DNA	ATP	36	1.9
+heparin		24	1.3
9 <i>EcoRK</i> +Ado-Met+ λ .0 DNA	ATP+heparin	800	41.8
		1,131	59.1

Set 1, Each reaction mixture of 250 μ l contained 0.13 μ g λ .0 32 P-DNA (14,650 c.p.m.), 6.7 nmol Ado-Met and 0.1 μ mol ATP. Sample 2 also contained 0.25 μ g heparin. *EcoRK* (0.1 μ g, 0.5 μ l) was added to the samples indicated and incubation at 30 °C was carried out for 2 min. The reaction was stopped by the addition of 50 μ l 0.5 M EDTA and filtered.

Set 2, Each reaction mixture of 5 μ l contains 27 pmol Ado-Met. In sample 5, 0.25 μ g heparin was also present. *EcoRK* (0.5 μ l, 0.15 μ g) was added, the samples were incubated at 30 °C for 3 min and diluted with 250 μ l of a reaction mixture containing 0.1 μ mol ATP and 0.13 μ g λ .0 32 P-DNA (14,650 c.p.m.) per 250 μ l or a similar mixture containing in addition 0.25 μ g heparin. After a further 2 min incubation the reaction was stopped with EDTA and the samples filtered.

Set 3, Procedure as for set 2 except that the λ .0 32 P-DNA was incubated with the 5 μ l enzyme activation mixture rather than added later during the dilution step.

heparin to a standard reaction mixture abolishes the binding of DNA to filters (Table 1, set 1). Heparin also inhibits the activated enzyme, both when it is added to the activation mixture, and when it is added to a dilution buffer containing DNA and ATP (Table 1, set 2). If, however, the enzyme is activated in the presence of λ .0 DNA and then diluted into buffer containing ATP and heparin, little inhibition is observed (Table 1, set 3). If heparin is added to a recognition complex, and ATP is added later, the same lack of inhibition is observed. Therefore, once the recognition complex has formed, the enzyme is no longer sensitive to inhibition by heparin. Normal DNA cleavage is observed when ATP and heparin are added to such a complex.

Both the initial and recognition complexes can be isolated on glycerol gradients

Direct evidence for the various enzyme-DNA complexes was obtained by glycerol gradient centrifugation. Sedimentation through glycerol gradients readily resolves DNA from the free enzyme (Fig. 4a, a composite of two different gradients). To detect the recognition complex, *EcoRK* was incubated with λ .0 32 P-DNA, Ado-Met and Mg^{2+} at 30 °C, chilled and sedimented through a glycerol gradient. Aliquots from each fraction were counted to determine the position of the DNA and further aliquots were assayed for the recognition complex (Fig. 4b). To detect the initial complex, the experiment was repeated with λ .K 32 HDNA in place of λ .0 DNA, and the gradient was assayed after addition of ATP and λ .0 32 P-DNA to an aliquot from each fraction (Fig. 4c). In both cases, the activated enzyme comigrated with the DNA. Surprisingly, a control incubation of enzyme with λ .0 32 P-DNA showed that the endonuclease was also bound to the DNA in spite of the fact that it had not been activated with Ado-Met (data not shown). This particular complex, however, does not seem to be an intermediate in the restriction reaction as the binding kinetics of this complex after ATP and Ado-Met addition are those of a standard reaction initiated by addition of *EcoRK* to λ .0 DNA and cofactors. As in the latter case, the reaction is inhibited by heparin.

An enzyme mechanism

Steps 1 and 2 of the reaction mechanism (Fig. 1) have been described in detail elsewhere⁸, and involve the fast binding of Ado-Met by the enzyme followed by a slow transition to an activated form.

Here, we are primarily concerned with the phenomenon of DNA recognition which we show involves the sequential formation of at least three different types of enzyme-DNA complex. To understand this process, we must briefly describe the relationship between the sites on the DNA for host specificity, methylation and cleavage. The recognition sites are those sites on the DNA that confer susceptibility to the endonucleolytic action of the enzyme. They are relatively few in number for a given DNA and may be lost by mutation to yield a DNA molecule that cannot be cleaved, *in vitro* or *in vivo*, by the enzyme⁸. The methylation site seems to be a specific sequence of bases at the recognition site, since phage mutants that have lost their recognition sites also lose (again, both *in vivo* and *in vitro*) the ability to be methylated^{9,10}. Furthermore, Horiuchi *et al.* have isolated small, specific DNA fragments from modified f1 DNA and found that of these fragments, only the two that contain the genetically mapped sB sites were methylated¹¹. The situation concerning the cleavage sites is less clear. The enzyme does not cleave the DNA at the recognition sites and the number of possible cleavage sites is larger than the number of recognition sites¹². Mapping studies with PM2 DNA have shown that the K cleavage sites are related to the readily denaturable regions of the genome (R.Y., T.A.B., W.E., and C.B., unpublished).

The interaction of *EcoRK** with the host specificity site presents one of the most interesting aspects of the reaction mechanism because it is this interaction which will determine whether the enzyme will methylate or cleave the DNA. The site can exist in three possible forms: fully modified, fully unmodified or heteroduplex (one strand modified, the other not). We have previously shown that such heteroduplex DNA is not a substrate for restriction¹, and Vovis *et al.*¹³ have shown that it is the best substrate for modification and that Ado-Met, ATP and

Mg²⁺ are required for optimal reaction. It is thus the recognition site that determines whether the enzyme will restrict or modify. If the site is unmodified, it will trigger the enzyme to its restricting mode, the enzyme will proceed to a cleavage site and restrict the DNA. If it is modified on one strand, the enzyme will be altered to its modifying form, and methylate the other strand. If it is fully modified, the enzyme will not recognise it, and will continue to scan other DNA molecules. The interactions of the enzyme with heteroduplex DNA are currently the object of extensive study in our laboratory. Both the recognition and cleavage complexes protect a small segment of DNA from DNase digestion. Sequencing of these fragments is currently in progress, and should clarify the location on the DNA of the recognition and cleavage complexes.

The restriction enzyme first breaks one strand followed some time later by a break on the opposite strand¹. At this point, it no longer behaves as an enzyme, since having cleaved once, it does not do so again. Simultaneously with DNA cleavage, a vigorous ATP hydrolysis occurs, which continues for a long period of time². Both observations can be readily reconciled if the restriction enzyme dissociated during cleavage to yield a species that would remain bound to the DNA. This complex would catalyse the ATP hydrolysis. Three observations lend support to this view: detection of a stable protein-DNA complex that hydrolyses ATP³; the lack of turnover (measured by both DNA binding and cleavage); and the complementation of a limit digest by either of two mutant restriction enzymes⁵ to give a new round of endonuclease activity.

In conclusion, it is clear that the mechanism of *EcoRK* is highly complex. What is not readily apparent is the reason for such an elaborate machinery to carry out reactions that are carried out in a much simpler manner by other restriction and modification systems. It cannot be ruled out that the complexity may be related to its involvement in other reactions such as DNA replication or recombination.

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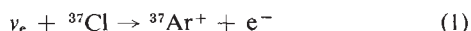
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letters to nature

Chemistry of the solar neutrino problem

ELECTRON neutrinos with energies of about 1-10 MeV are generally presumed to be produced by thermonuclear reactions in the Sun's core. Since 1967 an experiment has been under way on Earth to detect a subset of these neutrinos: argon atoms from the reaction



are chemically isolated, and the Auger electrons characterising their electron-capture decay are counted.¹ A sizeable discrepancy—the solar neutrino problem^{2,3}—has appeared. The most recent observational results⁴ imply an upper limit (1 σ) of 1 SNU (= 10⁻³⁶ solar neutrino captures per target ³⁷Cl nucleus s⁻¹), whereas the latest theoretical expectation based on standard models⁵ is 5.6 SNU.

Something is definitely awry in our understanding of solar structure theory, nuclear physics, neutrino physics, or the detection chemistry. A solution to the solar neutrino problem has been extensively sought in the first three of these areas^{6,7} (see also refs 2 and 3), but these efforts have recently encountered severe constraints⁸⁻¹⁰. The last area—the chemistry of the detection experiment—is one of the more neglected aspects of the problem; here I shall attempt to rectify this imbalance.

In the solar neutrino experiment a tank of liquid tetrachloroethylene (C₂Cl₄) provides the target ³⁷Cl nuclei of equation (1). The number of ³⁷Ar nuclei in the tank, *N(t)*, is governed by the equation

$$dN/dt = R + b - (N/\tau) \quad (2)$$

where *R* and *b* are the respective production rates resulting from solar neutrinos and background processes, and where τ (=50.6 d) is the electron-capture-decay lifetime of ³⁷Ar. For $t \gg \tau$, the solution to equation (2) asymptotically approaches the limiting value, $N(\infty) = (R + b)\tau$. The detector parameters¹ yield $R\tau = 9.6$ ³⁷Ar nuclei per SNU; the cosmic-ray-induced background for the water-shielded tank is estimated¹¹ to be $b = 0.09$ ³⁷Ar nuclei d⁻¹. Therefore

$$N(\infty) = 9.6(\text{SNU}) + 4.5 \quad (3)$$

where (SNU) denotes the actual capture rate of solar neutrinos in the tank of C₂Cl₄.

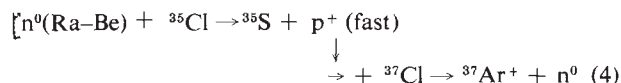
Standard theory⁵ implies that (SNU) $\simeq$ 5.6, so the anticipated steady-state population from equation (3) is $N(\infty) \simeq 58$ ³⁷Ar nuclei. The experimenters are attempting to extract fewer than 100 ³⁷Ar atoms from 390,000 l of tetrachloroethylene, to count them, and to determine the number produced by solar neutrinos in comparison with that arising from background processes. The complexity and marginal nature of the experiment lead me to put forward the hypothesis that the solar neutrino problem is solely an artefact of the chemistry of the detection technique. A direct corollary is that ³⁷Ar nuclei are, in fact, being produced by solar neutrinos in the tank at the rate of 5.6 SNU, so that $N(\infty) = 58$ ³⁷Ar nuclei. The experimental upper limit of 1 SNU then implies that only a small fraction of the ³⁷Ar in the tank is revealed in the output pulses of the Auger counter. (I shall use the loose terminology, 'chemical trapping', to indicate the fate of the undetected ³⁷Ar nuclei.)

Five experiments constrain this chemical hypothesis. First¹, a known volume of pure ³⁶Ar gas was dissolved in the tank

tetrachloroethylene, extracted by helium purging, and measured volumetrically; a 22-h extraction recovered 95% of the ^{36}Ar . Second (R. Davis, Jr, and J. C. Evans, Jr, unpublished), 612 ± 20 atoms of ^{37}Ar were mixed into the tank, the helium purge operated for three days, and the extracted ^{37}Ar detected in the Auger counter; it was inferred that the tank had contained 650 ± 50 (1σ) ^{37}Ar atoms.

These two experiments alone imply that neutral atoms of argon are not 'trapped' in Davis' system, even at the level of about 10^3 atoms. I note, however, that the product of reaction (1) is the cation $^{37}\text{Ar}^+$ and not neutral ^{37}Ar . The remaining three experiments probe the possible 'chemical trapping' of this charged $^{37}\text{Ar}^+$.

In the third experiment (see ref. 1), *in situ* irradiation of the tank liquid with fast neutrons produced $^{37}\text{Ar}^+$ by the reaction



Positive identification of ^{37}Ar at the Auger counter showed the helium-purge recovery of ^{37}Ar atoms to be proportional to that of the carrier ^{36}Ar , and the production rate was inferred to be 7.5×10^{-7} ^{37}Ar nuclei per incoming neutron. This determination was not, however, absolute; most of the ^{37}Ar nuclei could well have remained 'chemically trapped' in the tank.

The fourth 'experiment' (see ref. 11) was the calibration of cosmic-ray-induced ^{37}Ar in Davis' tank. The standard chemical procedure indicated that the equilibrium ^{37}Ar activity of small tanks of C_2Cl_4 at six different depths in the Homestake gold mine scaled with depth as expected theoretically; extrapolation to Davis' main tank implied a background rate of $b = 0.09 \pm 0.03$ ^{37}Ar nuclei d^{-1} . These results are, however, also not absolute, since the theoretical curve was normalised to the data at a depth of 25 hg cm^{-2} (standard rock). Should 'chemical trapping' prove important, then the canonical background rate will become highly suspect.

Ion-molecule reactions leading to the bound species $\text{ArC}_n\text{Cl}_m^+$ were tested in the fifth experiment¹², in which a gas-phase mixture of ^{40}Ar and C_2Cl_4 (at 1 mmHg pressure) was ionised with 40-eV electrons and the products were detected with a quadrupole mass analyser. No $\text{ArC}_n\text{Cl}_m^+$ was found, and the results were completely dominated by charge transfer (neutralisation of $^{40}\text{Ar}^+$ by electron pickup). To extrapolate these gas-phase findings to a liquid C_2Cl_4 environment ($\approx 10^6$ times denser), however, is totally unwarranted.

From this investigation of experimental constraints I conclude that the chemical hypothesis remains a viable solution to the solar neutrino problem only if the $^{37}\text{Ar}^+$ is 'chemically trapped' in Davis' system before neutralising to free ^{37}Ar atoms. This final loophole may be closed by another experiment now being performed by Davis (unpublished): a known number of $\text{C}_2\text{Cl}_3^{36}\text{Cl}$ molecules is put in his tank, the β -decay of ^{36}Cl produces a determinate number of $^{36}\text{Ar}^+$, and the amount of ^{36}Ar recovered is measured.

The evidence supporting my chemical hypothesis is purely circumstantial, but deserves mention. The clear signature of 58 ± 20 ^{37}Ar atoms in the tank at the end of run 27 was provided by the Auger counter^{4,13}; equation (3) then implies that $(\text{SNU}) = 5.6 \pm 2.0$. Contamination, cosmic-ray background variations, and statistical fluctuations are considered extremely unlikely causes^{4,14}, so the result of run 27 is generally attributed to a large $\bar{\nu}_e$ burst from a galactic 'collapsing star' (see refs 4 and 14; also R. Davis, Jr, and J. C. Evans, Jr, unpublished). On January 4, 1974 (during run 32), however, a strong $\bar{\nu}_e$ burst was apparently detected in an independent experiment in the Homestake gold mine¹⁵. If this burst signalled a 'collapsar', then a large accompanying $\bar{\nu}_e$ flux can be expected¹⁶, so it becomes difficult to understand the data of run 32 which implied only 9 ± 9 ^{37}Ar atoms in Davis' tank⁴. These observations can, however, be accommodated by my chemical hypothesis.

By what mechanisms may $^{37}\text{Ar}^+$ become 'chemically trapped' in Davis' system? Tetrachloroethylene is an unsaturated molecule, and short-chain polymerisation of liquid C_2Cl_4 has been achieved¹⁷ using MeV- γ -radiation; the $^{37}\text{Ar}^+$ may induce polymerisation and trap itself within a solid polymer analogous to Teflon. The rare-gas atoms, krypton and xenon, occur in stable molecules¹⁸ (for example, XeF_6), apparently partially because of electron-d-orbital interactions; since Ar^+ is isoelectronic to neutral chlorine there are 3d orbitals available which may permit 'back-binding', as in ClO_4^- and in the ' π -d-complex intermediates'¹⁹. The existence of stable molecules containing argon has been suggested²⁰. Finally, the stabilisation of ion-molecules involving Ar^+ and C_2Cl_4 (in bound excited states?) should be promoted by the strong inductive effects of Ar^+ and by cooperative relaxation processes in the liquid tetrachloroethylene environment. Chemical experiments to investigate some of these possibilities are currently underway.

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Ultraviolet laser sounding of the troposphere and lower stratosphere

WE report here measurements made with a ground-based, ultraviolet laser operating in the wavelength range 297-308 nm, which relate to the question of the atmospheric transmission between ground level and about 20 km, and particularly to the contributions of absorption by minor constituents. There has been considerable concern in recent years about the biological effects of increased solar ultraviolet radiation at ground level associated with a reduction in the concentration of atmospheric ozone¹. It seems that wavelengths between about 300 and 310 nm are most likely to produce erythema (sun burn) and skin cancer². The effects of aerosols, clouds, non-absorbing haze or fog and other absorbing atmospheric gases need to be considered in addition to the effects of ozone and of Rayleigh scattering when examining the penetration of these radiations to the Earth's surface^{3,4}. Except for the Junge layer⁵ of aerosols which extends from about 12 to 22 km, and cirrus clouds,

these scattering and absorbing agencies are probably located in the lower troposphere, below about 5 km.

Our observations were made at Winkfield (60 m above sea level and 40 km west of central London) with a laser radar system (Table 1). The transmitter consisted of a flash-lamp-pumped dye laser, tuned with Fabry-Perot etalons, frequency-doubled by an ammonium dihydrogen phosphate (ADP) crystal orientated for critical phase-matching. The receiver used a 1-m aluminised mirror, various interference filters and a bialkali-cathode photomultiplier. The absence

Table 1 Parameters of the ultraviolet laser radar system

Transmitter	
Wavelength range	297–308 nm
Linewidth	0.2 nm
Energy per pulse	approximately 0.2 mJ
Beam divergence	0.2 mrad $\times$ 2 mrad
Pulse duration	5 μ s
Pulse repetition rate	1 s ⁻¹
Receiver	
Mirror area	0.6 m ²
Beamwidth	2 mrad
Separation from transmitter	4 m

of spurious signals from any photomultiplier overloading or signal-induced noise⁶ was confirmed from soundings of cloud observed in the 5–10 km height range, above which no signal was detected. Rotation of the ADP crystal from the index-matching angle showed that the blocking of the receiver filters was sufficient to reject radiations scattered from the fundamental beam.

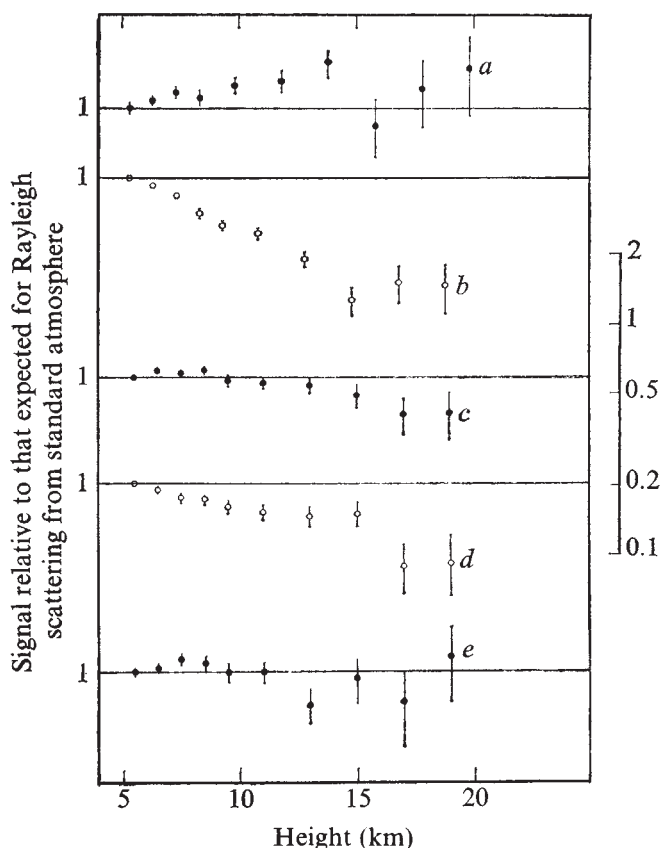
Figure 1 shows the results obtained on five nights when the laser was tuned to 308 nm. Measurements were restricted to periods when the sky was apparently clear, and the number of laser firings each night varied between about 100 and 1,000. The signal observed in each height channel was compared with that expected for Rayleigh scattering from the standard atmosphere for midlatitude winter conditions⁷, the resulting relative signal being normalised at 5.5 km. Attenuation of the beam because of Rayleigh scattering was allowed for in the calculation of the expected signal. The results obtained on different nights show height variations which are either consistent with Rayleigh scattering (February 24, 1975; Fig. 1e), show decreases with increasing height by up to a factor of 4 (January 12, January 31 and February 1, 1975; Fig. 1b, c, and d, respectively), or show small enhancements (December 30, 1974; Fig. 1a). Changes in the total molecular density cannot account for the night-to-night variability, so the results indicate either appreciable scattering and absorption by aerosols in the height range 5–20 km, or absorption of the radiation by a minor molecular constituent. This would probably be ozone, as the concentrations of sulphur dioxide, hydroxyl and other absorbing constituents above 5 km are expected to be too low to give detectable attenuation.

In an attempt to measure the height distribution of ozone, observations were also carried out using two wavelengths: 308.0 nm and 303.5 nm. These correspond to a ratio of 1 : 1.7 in the ozone absorption cross section^{8,9} and are close to minima in the sulphur dioxide cross section¹⁰. As the scattering and absorption characteristics of aerosols are not expected to vary significantly over this wavelength range, the difference in attenuation would arise from absorption by ozone. The results obtained are shown in Fig. 2 where the points for each wavelength have been separated horizontally for ease of presentation. For heights between 5 and 15 km, the two sets of results are each consistent with a straight line variation on the logarithmic scale. The ratio of slopes of these lines is closer to unity than would be

expected for absorption by ozone alone, but the difference in slope is found to correspond to ozone concentration of about 4×10^{17} m⁻³, a height-independent which is within the range of measurements by other methods¹¹.

Signals received from heights below about 5 km could not be interpreted, mainly because of uncertainty in the overlap of the transmitting and receiving beams. An estimate of the transmission coefficient below 5 km after allowing for Rayleigh scattering can, however, be obtained by comparing the observed signal from 5–6 km with that calculated from a knowledge of the ultraviolet pulse energy and overall receiver efficiency. A comparison of the results obtained for different wavelengths is relatively free from the uncertainties associated with the transmission coefficient obtained for each separately. From measurements carried out with a greater range of wavelengths on February 24, it was found that the two-way transmission coefficient below 5 km at 297 nm was lower by a factor of 10 than that at 308 nm. This cannot be explained by the attenuation effects of aerosols alone and absorption by molecular oxygen is negligible as the cross section is less than 10^{-29} m² (ref. 12). If the ratio of transmission at 297 and 308 nm is attributed to absorption by sulphur dioxide in the 0–1 km height range, a concentration of 4×10^{19} m⁻³ is implied (1 p.p.m.), which is very unlikely. If absorption by ozone in the height range 0–5 km were responsible, an average concentration of about 5×10^{18} m⁻³ would be required. This is an order of magnitude greater than that deduced for the 5–15 km height range, and is about five times larger than the mean background concentrations observed near the ground in north-western Europe during summer and is approached only during periods of photochemical air pollution^{13,14}. The

Fig. 1 Observed signals at 308 nm on five nights between December 1974 and February 1975, relative to those expected for Rayleigh scattering: a, December 30; b, January 12; c, January 31; d, February 1; e, February 24. Error bars indicate the standard deviations corresponding to the photon count for each height channel.



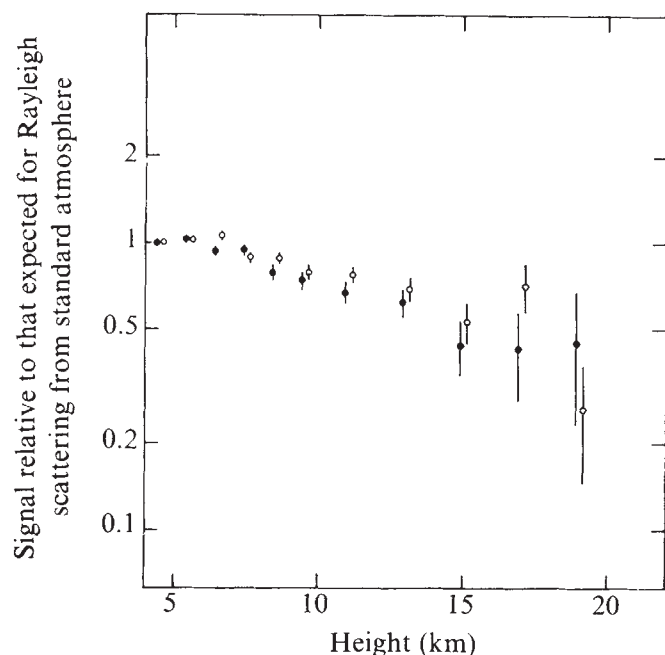


Fig. 2 Observed signals at 303.5 nm (●) and 308.0 nm (○) for February 27, 1975, relative to those expected for Rayleigh scattering. Error bars indicate the standard deviations corresponding to the photon count for each height channel.

results can be better interpreted in terms of absorption by water vapour in the height range 0–5 km; a difference in absorption cross section of about 10^{-27} m² between 297 and 308 nm would be required for that to occur, but no accurate measurements of cross section are available for this range of wavelengths.

There is considerable scope for the further development of this technique of ultraviolet laser sounding of the atmosphere. With the production of new frequency-doubling materials suitable for 90° phase-matching¹⁵, it should become possible to increase substantially the available ultraviolet pulse energies. The accuracy and height resolution of the present type of measurement should thereby be improved, and the height range extended. In addition, measurements of the concentrations of certain constituents by resonance scattering or fluorescence should become possible.

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Petroporphyrins as indicators of geothermal maturation

WE report here two new sets of data which provide evidence of the occurrence of the postulated conversion of deoxyphylloerythroetioporphyrin (DPEP) to etioporphyrin. Simulated geothermal maturation in the laboratory demonstrates that DPEP–etio conversion occurs proportionally to the severity of the treatment, and an analysis of petroporphyrins from a suite of genetically related petroleum and their source rocks reveal that the DPEP:etio ratio is inversely proportional to the presumed severity of the geothermal history of the sample.

Alkyl petroporphyrins occur in a wide range of geological materials^{1,2} where they are usually present as two major homologous series of the deoxyphylloerythroetioporphyrin (DPEP) and etio type. Initially, the DPEP compounds containing the isocyclic ring were thought to be representative of, and derived from, chlorophylls, whereas the etio homologues, with no isocyclic ring, were considered to be remnants of the haem type pigments³. But though chlorophyll pigments exist in far greater abundance than haem pigments in the biosphere⁴, DPEP and etio porphyrins⁵ frequently occur in similar amounts in geological samples, and Corwin suggested⁴ that the conversion of

Fig. 1 Relative abundance of metallopheoporphyrins (VO) from thermally altered (210 °C for 0 (a), 50 (b), 100 (c), 150 (d) and 385 (e) h) La Luna (Mara) extract obtained by mass spectrometric determination of M⁺ at the following masses: DPEP (solid line) = (457+n14) a.m.u.; etio (dashed line) = (459+n14) a.m.u.; where n = 1 to 13 (C₂₇ to C₃₉). The mass spectra were collected by computer controlled gas chromatography–mass spectrometry; because of instrument background low abundance ions are occasionally not recorded (for example, C₂₇, C₃₈, and C₃₉ in the 50-h (b) and 100-h (c) heating experiments).

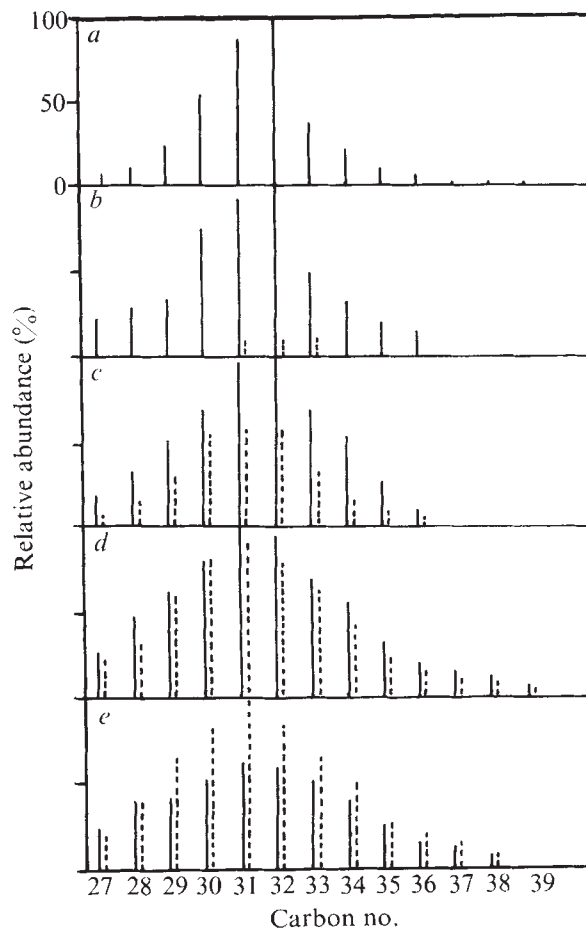


Table 1 Characteristics of petroporphyrins and source rocks from the Maracaibo Basin

Sample	Current geological setting					Petroporphyrins		
	Type	Age*	Depth (m)	Temperature (°C)	Content† (p.p.m.)	Carbon no. of most abundant molecular ion	DPEP	etio
a La Luna (Mara)	Source rock	Cretaceous	outcrop	< 30†	55,900	32	—	≥ 100
b Boscan (BN-4)	Petroleum	Eocene reservoir	2,050–2,230	71	3,540	32	31	1.56
c La Paz (P187-Z)	Petroleum	Jurassic fracture	3 300–3,450	95	628	31	30	0.56
d La Luna (36E-2)	Source rock	Cretaceous	4,410–4,550	135	2,730	31	30	0.56

*The two oils are believed to have originated in the La Luna Formation (Cretaceous) but are currently found in the host formations indicated¹³.

†No information concerning maximum depths of burial or temperature history is available but low reflectivity ($R_{av} = 0.19$) of vitrinite suggests a mild geothermal history. (We thank Dr M. Jones, Organic Geochemistry Unit, University of Newcastle, for this measurement.)

‡Metalloporphyrin content (p.p.m.) in oil or organic extract.

DPEP to etio homologues must occur in the geological environment. Indirect evidence of the conversion of DPEP to etio has been obtained from a comparison between petroporphyrins recovered from a retorted shale oil and those from the original shale⁶. The retorted oil contained a large proportion of etio type homologues, suggesting that conversion did occur during the retorting. The drastic temperatures required for the retorting process are, however, significantly higher than those (about 50–180 °C) normally experienced in deeply buried sediments, which effect the geothermal maturation of entrapped or dissolved petroporphyrins and other organic materials. Evidence of the conversion has also been provided by the thermal alteration, in the laboratory, of a crude petroleum containing metalloporphyrin to which etioporphyrin III had been added as an internal reference compound⁷. Other studies which have simulated geothermal maturation^{8–11} have been restricted to porphyrins of the etio skeleton; they could not, therefore, provide information about the fate of the isocyclic ring of DPEP homologues or about the occurrence of the DPEP to etio conversion.

The geological materials used (Table 1) in this study are from, or are genetically related to, the Cretaceous La Luna formation of the Maracaibo Basin in western Venezuela. This formation is extensive and source beds in it are believed to have generated the major part of the oil produced in the Maracaibo area¹². One of the most remarkable characteristics of these shaly limestones is the high porphyrin content (up to 55,000 p.p.m. of the organic extract); it has, in fact, been used to establish source rock–crude oil relationships for the oils from the Maracaibo Basin¹³.

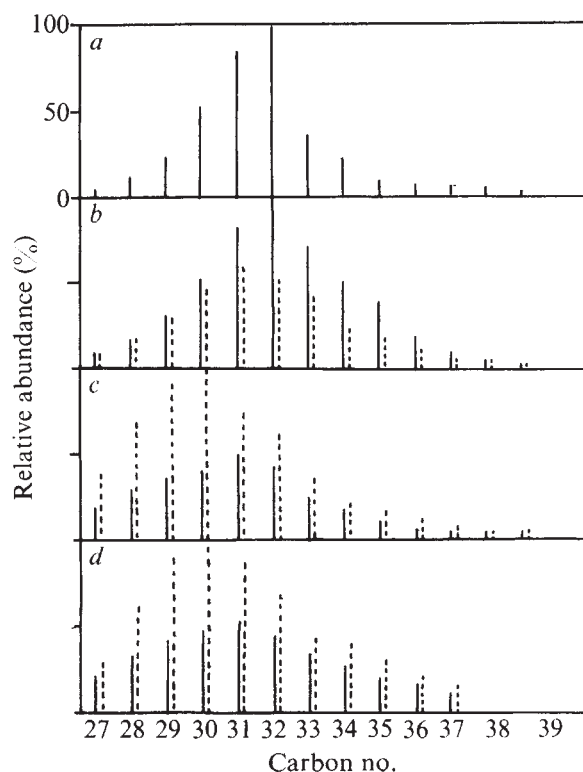
The simulated geothermal maturation was performed on the total organic extract of the least mature shale (Table 1a). The toluene–methanol (3:1) extract was deposited on prewashed (redistilled hexane B.D.H.) and vacuum-dried bentonite to give a concentration of about 2,790 p.p.m. of metallopetroporphyrin (98% vanadyl). Bentonite, a montmorillonitic clay mineral was chosen as the organic free matrix for thermal alteration experiments. Results obtained using this material rather than limestone should be more generally applicable to the geothermal fate of porphyrins in argillaceous sediments.

Aliquots of coated bentonite were placed in heavy-walled glass tubes (15 × 150 mm), flushed with oxygen-free nitrogen and sealed under vacuum. The sealed tubes were stored in the dark at 210 °C; individual tubes were removed at successively longer time intervals from 0 to 385 h. After cooling, the bentonite was extracted with toluene–methanol (3:1) and the metallopetroporphyrins were isolated, and purified using thin-layer chromatography (silica gel H, 0.5 mm, toluene–methylene chloride 1:1). Recoveries of total metallopetroporphyrins after thermal treatment varied from 81% (0 h) to 19% (385 h experiment). The thermal degradation of metallopetroporphyrins was found to be first order ($t_{1/2} = 166$ h). The extracts were examined using mass spectrometry: a computerised data handling system¹⁴ monitored and collected the spectra in real time.

Bar diagrams (Fig. 1) show the relative abundance of the molecular ions of metallopetroporphyrins isolated after various times of thermal treatment. The zero-time extract exhibits a single homologous DPEP series of carbon number C_{27} – C_{39} , maximising at C_{32} . After 50 h an etio series could just be detected, after 100 h this was prominent and after 385 h the abundance of this series exceeded that of the now much-diminished DPEP compounds. Thus, the DPEP series seems to have undergone extensive thermal conversion to the etio series. The most abundant DPEP homologue remaining was then C_{31} , indicating that a second maturational process involves loss of alkyl groups. Dealkylation is a well known feature of geothermal maturation of other geolipids¹⁵ and has been recorded following the laboratory thermal alteration of etioporphyrins^{8–11}.

In the second part of this study petroporphyrins from two crude oils and from the toluene–methanol (3:1) extracts of two

Fig. 2 Relative abundance of petroporphyrins isolated from geological samples described in Table 1. Mass spectrometric determination of M^+ occurring at the following masses: DPEP (Solid line) = $(392 + n14)$ a.m.u.; etio = $(394 + n14)$ a.m.u.; where $n = 1$ –13 (C_{27} – C_{39}). a, La Luna (Mara); b, Boscan BN4; c, La Paz P187-Z; d, La Luna 36E-2.



different samples of their presumed source rock (Table 1) were isolated using demetallation-extraction with methanesulphonic acid (4 h at 110°C) and were analysed using mass spectrometry¹⁶. Figure 2 presents the relative abundances of the petroporphyrins of these samples; the samples increasing in maturity (based on geological information) from top to bottom of the diagram. As the geothermal maturity increases there is an increase in the relative abundance of the etioporphyrins and a shift to the lower carbon number of the most abundant petroporphyrin observed (Table 1). The decrease in the DPEP : etio ratio would suggest that during geothermal maturation DPEP species are converted into etio homologues. A general trend observed¹⁷ within a group of non-genetically related geological materials suggests that the DPEP : etio ratio decreases with the depth of burial.

The DPEP-etio conversion suggested for the Maracaibo Basin samples parallels the conversion observed in the thermal alteration experiment. In each case the maxima of the two homologous series occur either at the same carbon number or with the abundance of etio homologues maximising one carbon atom lower than the DPEP homologues. Similar distributions have been observed in a wide range of naturally occurring petroporphyrins¹. The thermally induced conversion of DPEP to etio porphyrins may be widespread in the geological environment and could account for the abundance of etio homologues in crude oils and sedimentary rocks. The decrease in the DPEP : etio ratio with increasing maturation could be a useful measure of the extent of maturation of genetically related geological materials.

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or goethite (α FeOOH) to the ferrimagnetic form, maghaemite (γ Fe₂O₃). He also proposed two possible mechanisms involving a reduction process followed by reoxidation to maghaemite. In the first (fermentation mechanism), the reduction occurs as a result of the decay of organic matter in the soil in anaerobic conditions achieved during wet periods, and reoxidation to maghaemite occurs in the aerobic conditions prevailing during subsequent dry periods. In the second (heating mechanism) the burning of organic material produces the temperature increase and reducing atmosphere necessary for the reduction to magnetite (Fe₃O₄) in a thin layer of soil underlying the fire and reoxidation occurs during the cooling down of the fires when air enters the system.

On the basis of the heating mechanism, the percentage of the iron oxide in the soil which has been converted *in situ* to maghaemite (that is, the percentage conversion) is given by the ratio of the observed magnetic susceptibility (χ_o) to the susceptibility (χ_N) after heating the soil in a laboratory furnace at 550°C in an atmosphere of nitrogen followed by air³. Although pure maghaemite converts to haematite at temperatures above 350°C, maghaemite doped with, for example, sodium, aluminium or magnesium is stable up to very much higher temperatures⁴. Therefore, since the iron oxides in soils are in a finely divided form and in intimate contact with the clay minerals, it is assumed that the laboratory heating converts the iron oxides to a thermally stable, impure maghaemite. Support for this hypothesis has been provided by Le Borgne¹ who has shown that soils with a high magnetic susceptibility exhibit an exothermic DTA peak, characteristic of maghaemite, at about 700°C. Furthermore, X-ray diffraction measurements on the laboratory heated soils have established that either maghaemite or magnetite is present, and in view of the final oxidising stage in the heating, the latter alternative is unlikely.

Magnetic susceptibility measurements (Table 1) were undertaken, using an a.c. bridge system⁵ operating at 1 kHz, on soils (0-20 cm; A horizon) derived from a wide range of sedimentary rock deposits (Triassic-Miocene) in England and Italy, as well as on a small selection of soils from other parts of the Mediterranean zone and from various tropical regions.

Table 1 Magnetic susceptibility ranges for soils

Region	Geology	Magnetic susceptibility ($\times 10^{-8}$ kg ⁻¹)	
		Observed (χ_o)	After heating (χ_N)
England	Limestone	15-90	1,000-5,000
England	Sandstone, clay	10-180	600-8,000
Italy	Marly limestone, marl	10-80	600-3,000
Italy	Sandstone, clay	5-30	600-2,000
Italy	Limestone	30-500	200-4,000
Mediterranean	Limestone	150-600	1,500-3,000
Mediterranean	Calcareous alluvium	30-400	500-6,000
Tropics (wet and dry)		15-500	200-8,000
Tropics (rainy)		5-15	700-1,000

The percentage conversions (that is, $100\chi_o/\chi_N$) observed for the English soils were normally less than 5%, irrespective of the type of sedimentary rock from which they were derived (Fig. 1a). In contrast, the percentage conversions observed for the Italian soils differed widely depending on the associated rock-type. Soils developed on marly limestones, marls, sandstones and clays, again normally exhibited percentage conversions of less than 5% (Fig. 1b) whereas those developed on hard, as opposed to marly, limestones exhibited significantly higher percentage conversions extending up to 50% (Fig. 1c). These differences in percentage conversion cannot be satisfactorily explained in terms of the heating mechanism as it is extremely unlikely that the limestone soils from Italy have been consistently subjected to more extensive burning than either the other

Effect of climate on the magnetic susceptibility of soils

THE magnetic susceptibility of soils derived from sedimentary rocks is normally significantly higher than that of the parent rock. Le Borgne^{1,2} has suggested that this enhanced susceptibility of the soil is due to the *in situ* conversion of the iron oxides from an antiferromagnetic form such as haematite (α Fe₂O₃)

Italian soils or the English soils. Instead, it seems probable that the higher percentage conversions associated with the limestone soils from Italy arise because the fermentation mechanism is more extensively activated as a result of the combined effect of the prevailing Mediterranean climate and the high permeability of the limestone substratum. Anaerobic (reducing) conditions are achieved during the humid winters and the necessary aerobic (oxidising) conditions are achieved during the subsequent hot dry summers in the case of the well-drained soils which are developed on limestones.

Further evidence for the role of climate is provided by the fact that the limestone soils from Apulia, the Orbetello region and the southern Apennines, which are mainly of the red Mediterranean type⁶ (that is, terra rossa) exhibit higher percentage conversions than those from the central Apennines which are mainly rendzinas (Fig. 1c). Again, it seems probable that this difference is because more sustained aerobic conditions are achieved during the summer months in the former areas as a result of lower rainfall and higher temperatures.

The percentage conversion data for soils developed on limestones and for calcareous alluvia from Greece, Crete and Turkey (Fig. 1d) confirm that high values for this parameter are not peculiar to Italy but seem to be generally characteristic of well-drained soils in the Mediterranean climatic zone. Similarly, the data for tropical soils from Nigeria, Gambia, Zambia, British Honduras and North Borneo provides further evidence for the role of climate in determining the percentage conversions achieved in soils (Fig. 1e). The majority of the soils from those regions in which a pronounced dry period occurs (that is, the

wet and dry tropics) again exhibit percentage conversions in excess of 5%, the characteristic red tropical soils being predominant in this group. In contrast, the soils from those regions in which no definite dry period occurs (that is, the rainy tropics) exhibited percentage conversions of less than 2%.

These results suggest that there is a high percentage conversion of iron oxide to the ferrimagnetic form, and a concomitant high magnetic susceptibility (Table 1) in well-drained soils developed in regions with a climate that includes a pronounced, hot, dry period. It is, therefore, possible that magnetic susceptibility measurements could be used to identify those palaeosols in, for example, England which were formed in warmer dryer climatic conditions than exist at the present time. In interpreting the magnetic susceptibility of soils it must, however, always be remembered that high percentage conversions can also be produced by burning associated either with the clearance of land for agriculture or with human habitation. For example, in England, although normal soils exhibit low percentage conversions (Fig. 1a), soils from archaeological sites (that is, topsoils, pit and ditch fillings) exhibit significantly higher percentage conversions in the range 4–20% (ref. 7).

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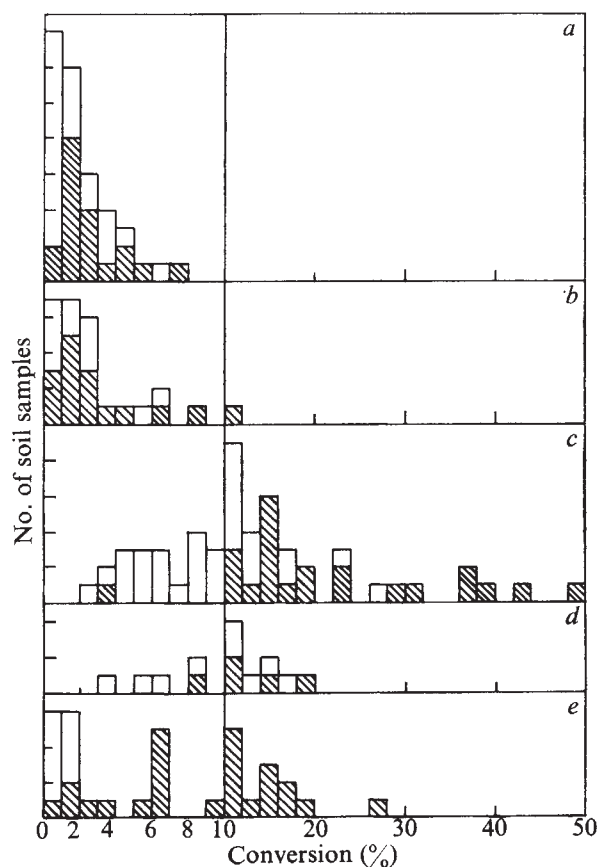
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Fig. 1 Histograms showing the distribution of values for the percentage conversion for: a, English soils derived from limestones (shaded), and sandstones and clays (blank); b, Italian soils derived from marly limestones and marls (shaded), and sandstones and clays (blank); c, Italian soils derived from limestones in Apulia, the Orbetello region and the southern Apennines (shaded), and the central Apennines (blank); d, Mediterranean soils derived from limestones (shaded) and calcareous alluvia (blank); e, soils from wet and dry tropics (shaded) and rainy tropics (blank).



New interpretation of dielectric loss peaks

ALTHOUGH the dielectric loss peaks in many materials are known to show a frequency dependence which departs radically from the ideal Debye shape, the interpretation of their temperature dependence is carried out implicitly within the framework of the Debye mechanism. This cannot produce a good physical insight into the situation and here I look at it from a new standpoint.

The frequency dependence of loss, $\chi''(\omega)$, in many dipolar materials may be expressed by the empirical formula^{1,2}:

$$1/\chi''(\omega) = (\omega/\omega_2)^{-m} \pm (\omega/\omega_1)^{1-n} \quad (1)$$

where ω_1 and ω_2 are generally, thermally activated parameters, and the exponents m and $(1-n)$ are both smaller than unity and decrease with decreasing temperature. This law of reciprocal addition gives a loss peak at a frequency ω_p , which increases with temperature. In many dielectric materials in which the polarisation is caused by hopping charges rather than by dipoles the peak is not readily observable because of the onset of direct current conduction, but the loss is still given by the second term in equation (1). I shall refer to this as the 'universal law of the dielectric behaviour'.

This law implies that the ratio of the imaginary to the real parts of the susceptibility is independent of frequency

$$\chi''(\omega)/\chi'(\omega) = \cot(\pi/2) \quad (2)$$

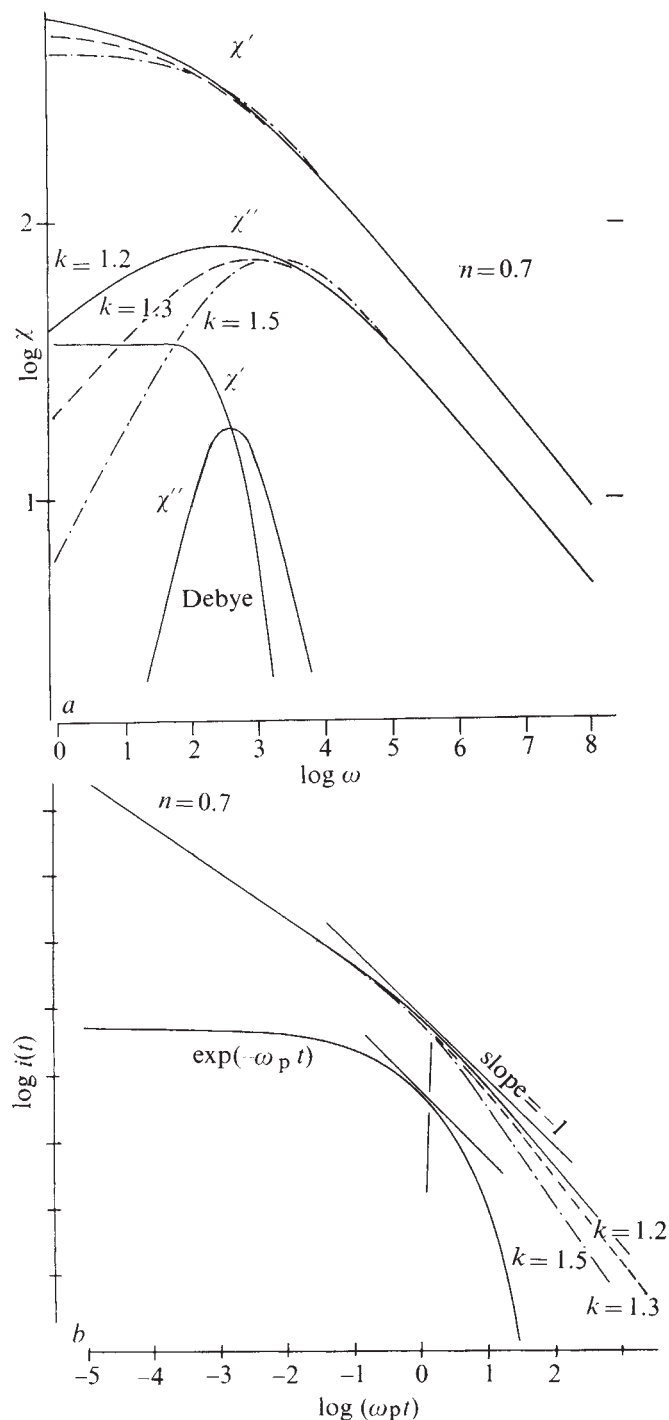


Fig. 1a Frequency-dependence of loss; b, time-dependence of current under step-function electric field excitation. Both correspond to equation (4), with $n = 0.7$ and values of $k = 1.2, 1.3$ and 1.5 . The $\chi'(\omega)$ and $\chi''(\omega)$ curves are Fourier-transformed numerically from equation (4) and show the parallel slope on the high-frequency side of the peak and the effect of the exponent on the positive slope and on the half-width of the peak. The pure Debye characteristics are shown on the same frequency scale in a and the exponential response on the same time scale in b. The vertical scales in a and b are scaled by arbitrary factors.

and leads to the physical interpretation that the ratio of energy lost per cycle to energy stored per cycle is constant, in marked contrast to the Debye mechanism for which it is equal to $\omega\tau$, where τ is the appropriate relaxation time. Any model satisfying this energy criterion must necessarily follow the universal law as a consequence of the Kramer-Kronig relationship. A physical mechanism which has the required properties and which should

be very widely applicable to both dipolar and hopping charge systems requires two assumptions: first, that charges or dipoles do not move smoothly but in discontinuous hops or jumps between preferred positions or orientations; second, that interactions with other charges or dipoles give rise to partial screening which can only follow the abrupt transitions with a finite delay.

The second term in equation (1) has, therefore, a simple interpretation in the frequency domain. The physical significance of the first term is less clear but I have suggested³ that the gradual decrease of loss as $\omega \rightarrow 0$ arises because the perturbation of the system from its thermal equilibrium condition becomes progressively slower.

In the accepted interpretation of the temperature dependence of ω_p , the loss peak is treated as though it were a simple Debye peak⁴. The fact that its width at half-height may be two to eight times that of a Debye peak is left out of consideration, even though it may be acknowledged that the shape, and therefore the position of the peak, is determined by some distribution function, $g(\tau)$, of Debye relaxation times.

The concept of $g(\tau)$ is superficially very plausible—there is no doubt that physical systems must contain distributions of parameters rather than show strictly discrete values, especially in disordered materials and where stochastic processes govern the behaviour. In certain simple systems—for example, those involving multiple allowed orientations of molecular dipoles⁵ or systems in which localised electrons execute multiple hops (V. Halpern, private communication)—it is possible to calculate exactly the distributions involved. Very complicated situations may arise for such cases as polar sidegroups on folded polymeric chains, but no rigorous analysis is possible in those cases⁶.

What seems to have happened with the passage of time, however, is that the otherwise plausible, if restricted, concept of $g(\tau)$ became the universal explanation of all phenomena which did not fit into the idealised pattern, thus acquiring gradually the status of an unquestionable truth. The first point I challenge, therefore, is the scientific justification of the concept of the distribution of relaxation times in its application to the interpretation of dielectric data.

My argument is based on the universality of the observed behaviour^{1,2} covering materials from plastics, through glasses, metal oxides, organic long-chain molecules and on to sand aggregates—seemingly regardless of their physical structure, chemical combination and bondage, or of the nature of the prevailing charged species responsible for polarisation (for example dipolar, electronic or ionic). It is completely implausible that the distribution of relaxation times in all of those diverse systems should happen accidentally to be such as to give the observed behaviour provided by the second term in equation (1). Having proposed a physical mechanism for the interpretation of this part of the spectrum¹, I turn here to the physical mechanism governing the region of the loss peak and below.

The precise form of the first term in equation (1) is of little significance here and the essence of my argument is not affected by it.

I propose that a more direct insight into the physical nature of the loss peaks may be obtained from an analysis of the time-domain response, $i(t)$, of a dielectric to step function electric field excitation. For an ideal Debye system:

$$i(t) \propto \exp(-t/\tau) \quad (3)$$

and the response corresponding to the empirical law is:

$$i(t) \propto [(t\omega_p)^n + (t\omega_p)^k]^{-1} \quad (4)$$

where the exponent, n , is the same as in equation (1) and $k > 1$.

Figure 1 shows a set of corresponding ω -domain and t -domain responses for an ideal Debye system and for the empirical behaviour, with $n = 0.7$ and with three values of k : 1.2, 1.3 and 1.5, respectively. In the latter case, ω_p corresponds to the time at which the slope of the $\log \chi'' - \log \omega$ graph changes from a

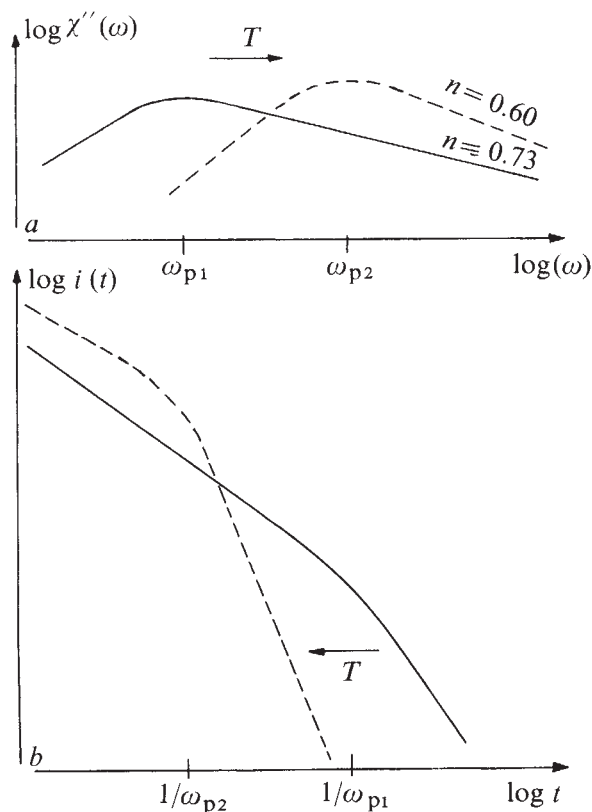


Fig. 2 Schematic representation of the effect of a rise in temperature: a, on $\chi''(\omega)$; and b, on the corresponding $i(t)$. The slopes m and $(1-n)$ in a reflect qualitatively the observed behaviour. Solid lines, lower temperature; dashed lines, higher temperature.

value of less than unity to one of more than unity; that is, at the 'kink' in the response curve. Similarly, for the Debye mechanism the peak frequency is associated with the time, $t = \tau$, at which the slope goes through -1 . But whereas the Debye exponential relationship (equation (3)) gives a smoothly varying slope corresponding to a single physical process in which the polarisation $P(t)$ tends exponentially to the limiting steady-state value, P_0 , the empirical relationship is clearly seen to correspond to distinct physical processes. This implies that the polarisation process occurs in two separate stages with transition at a time $t = 1/\omega_p$.

I suggest the following physical significance of these two stages in terms of the mechanism characterised by the two points already mentioned. At time $t < 1/\omega_p$ after the application of the step function field the response $i(t) \propto t^{-n}$ is determined by a 'primary' adjustment of dipoles or hopping charges to the prevailing field, accompanied by the corresponding delayed screening processes. At time $t = 1/\omega_p$ the primary adjustments have been substantially completed and a 'secondary' process sets in, giving the final adjustment of screening and producing the observed, more rapid rate of change of current at times in excess of $1/\omega_p$.

The loss peak in the frequency domain is, therefore, seen as a manifestation of the onset of the secondary processes in the time-domain response which are implicit in the non-Debye model. The temperature dependence of ω_p arises from a shift of the demarcation time towards smaller values with increasing temperature, which may be seen as a result of the primary processes occurring more rapidly. The secondary processes also become more rapid, leading to the observed higher values of the exponent, m , in equation (1)^{1,2} as shown in Fig. 2.

I conclude that the loss peak in solid dielectrics arises from an interaction between two separate processes, which may be described as the primary and secondary relaxations in an

interactive assembly. In this interpretation it seems that the determining factor is the duration of the primary process, after which time the secondary process takes over. This time is thermally activated with a well defined energy and so, therefore, is the peak frequency, ω_p .

It is not surprising that the loss peak is not caused by a single 'homogeneous' process as in the Debye model. Even in the accepted interpretation through $g(\tau)$ it is the shape of the distribution function and not the frequency dependence of any particular Debye process that determines the loss peak. The physical significance of $g(\tau)$ has never been made very clear but there is no reason to assume that the rising and falling parts of it are determined by a single physical process.

The foregoing discussion is essentially qualitative and its main object is to point towards a new approach to the old standing and unresolved problems of dielectric relaxation. The justification for this qualitative approach is twofold: the very generality of the phenomena that must be interpreted and the lack of rigour in the accepted point of view. There now remain the difficult tasks of developing a more quantitative approach and also of finding critical experimental evidence against which to test the proposed theory.

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Evolution of Proterozoic basement patterns in the Lewisian Complex

Two contrasting structural elements, both of which have affinities with the two Proterozoic mobile belts of Greenland, have contributed to the pattern of structures in the Lewisian complex of the northern British Isles. The evolution of these structural patterns can be interpreted in terms of an integral system of movements which developed under the influence of an evolving linear dislocation that flanked the major Archaean craton of Greenland.

Allowing for continental drift, the Precambrian basement terrains of the North Atlantic Province (Greenland, Labrador and north-western Britain) outline an Archaean craton about 600 km across, which became stabilised about 2,500 Myr ago. Two major Proterozoic mobile belts apparently related to others in the neighbouring Canadian province, border this North Atlantic craton (see ref. 1): to the north, the Nagssugtoqidian, characterised by crustal shortening perpendicular to the belt², high pressure regional metamorphism and limited igneous activity; and to the south, the Ketilidian, in which vertical or transcurrent displacements, low pressure metamorphism and widespread magmatic activity occurred. The continuations of these belts may intersect in the Lewisian of north-western Britain². The Lewisian comprises a number of small stable blocks of the order of 10 km across composed of Archaean rocks showing relatively slight, late modifications (Scourian complexes) set

in a framework of Proterozoic structures (Laxfordian complexes). North-westerly and north-easterly trending structures within this framework impart the characteristic Proterozoic tectonic 'grain' to the Lewisian, although north-easterly trending Proterozoic structures have only been described in northern Lewis³.

The occurrence of linear structures along the margins of regions which have been little affected by Proterozoic reworking is a characteristic of north-westerly trending Proterozoic belts in the Lewisian. Using Archaean basic igneous complexes as marker horizons, it is possible to identify large-scale isoclinal folds with north-westerly trending axial surfaces, which have existed in the region south of Loch Laxford since at least the end of Archaean granulite facies metamorphism (about 2,800 Myr ago). Other north-westerly trending structures, such as those of the Ness region, Lewis, also seem to have begun to develop in pre-Laxfordian times^{3,5}. This suggests that Lewisian structures with north-westerly trends have evolved on, or are related intimately to, a series of lineaments which originated during the late Archaean. Discounting local variations in finite strains, both the Laxford and Ness regions were sites where strain trajectories in the *xy* plane (axial surfaces and schistosity) had gentle to moderate dips and where displacements occurred obliquely to, but effectively perpendicular to, the north-westerly trending lineaments immediately before and after the emplacement of the dolerite Scourie Dykes⁷. At Laxford, movements on shear zones seem to have resulted in local crustal shortening by the overthrusting of Scourian granulites on to remobilised migmatites⁸. Throughout much of the Lewisian of the mainland and the Isle of Lewis, the regions occupied by flat lying, early Laxfordian structures and tectonite fabrics have suffered moderate to high early Laxfordian strains which were apparently caused by large scale, horizontal, ductile shearing.

By late Laxfordian times, however, the Ness region was the site of steep *xy*-plane strain trajectories, suggesting displacements around the margins of a more competent block of central Lewis⁷. Similarly, at Laxford, a zone occupied by late Laxfordian granite sheets developed as a steep shear zone⁶ parallel to the north-westerly trending lineament.

A north-easterly trending zone of steep dipping foliations lies along western Lewis and intersects the north-westerly trending structures of Ness, in Lewis. This steep zone was probably in existence in pre-Laxfordian times³ and persisted as a discrete zone of steep foliation compatible with displacements parallel to the margin of the lineament into the late Laxfordian⁷. A similar north-easterly trend has been recognised on geophysical evidence, and is attributed to variations in rock densities, which originated in pre-Laxfordian times⁹; it is also associated with the widespread occurrence of successive groups of late Laxfordian granitic intrusions along the western seaboard of Harris and Lewis.

This arrangement of structures suggests that two systems with contrasting strain trajectories and contrasting major displacements, have acted contemporaneously throughout much of the Proterozoic. Although most influence seems to have been exerted by the zones within which deformation with shallow-dipping *xy*-plane trajectories and displacements mainly perpendicular to the north-westerly trending lineaments occurred during the early Proterozoic, steeply-dipping *xy*-plane trajectories and displacements parallel to the strike were established in the north-easterly trending structure at the same time as displacements on shallow dipping movement planes occurred. Finally, steeply-dipping *xy*-plane trajectories were established parallel to the lineaments in both north-easterly and north-westerly trending structures. Most probably, major discrete dislocations which behaved in a more ductile manner and which separated less ductile 'stabilised' regions, became

more influential during the later stages of Proterozoic evolution in both the Lewisian³ and in eastern Greenland¹⁵.

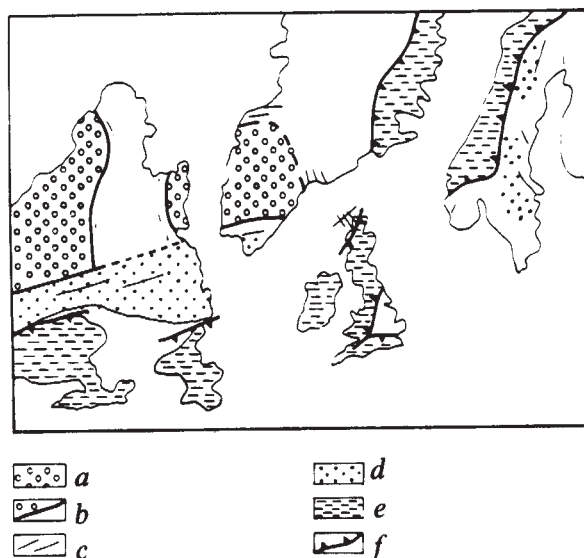
Mineral parageneses indicate that a change occurred from the high pressure environment that existed in the Scourian and early Laxfordian¹⁰, to a low pressure environment during the mid to late Laxfordian^{11,12}. This off-loading of confining pressure was probably connected with the change from the sub-horizontal crustal shortening (conceivably entailing at least local crustal thickening) that characterised the early Proterozoic to the sub-vertical zones of displacement that dominated the late Proterozoic.

Although it is clear that no single directional element in the tectonic pattern of the Lewisian could be compared directly in scale or behaviour with any individual belt of Greenland, it seems that the Proterozoic patterns in the Lewisian complex are the results of the integration of two nearly contemporaneous but contrasting evolutionary processes somewhat resembling those recorded in separate parts of the adjacent area of Greenland.

In the Lewisian, the north-westerly trending lineaments are distinct sites within which metamorphism and magmatic activity were often directly related to tectonic movements¹⁶, whereas in the north-easterly trending lineaments metamorphism and magmatic activity seem only to be related in a general way to tectonic activity^{3,9}. The relatively large, gently dipping zones within which ductile shearing occurred perpendicular to the lineaments under high pressure and temperature conditions during the early stages of development of the Lewisian belts can loosely be compared with the dominant displacements in the Nagssugtoqidian mobile belt². In contrast, the zones of near vertical displacements, relatively low confining pressures and successive granite intrusion, which occurred in both regions during the late Laxfordian could be compared with the dominant regime of the Ketilidian mobile belt.

Since sets of structures compatible with both the major belts of Greenland intersect in the Lewisian, it may be that the two major belts of Greenland simply cross in the Lewisian². But although the Ketilidian seems to be part of a major belt of Proterozoic reworking extending from the south-western United States, around the Canadian province, to the Urals¹⁴, there is little evidence of any continuation of the Nagssugtoqidian Belt (Fig. 1). Alternatively, both the

Fig. 1 The Lewisian in the context of Proterozoic structures from Labrador to Scandinavia. *a*, Archaean craton; *b*, Proterozoic lineament; *c*, tectonic trend lines; *d*, regions of Proterozoic magmatism; *e*, Palaeozoic mobile belts; *f*, Palaeozoic orogenic front.



Greenland belts could have suffered displacements similar to those undergone in the Lewisian and it could be that only the dominant set in each belt has so far been recognised. Evidence which might support this suggestion comes from the intersections between tectonic trends in the mobile belts and major dislocation lineaments which border the cratons of Canada, and indicate a sense of movement on major dislocations similar to those in compatible dislocations in the Lewisian¹³. This interpretation of systems of major dislocations indicates the significance of the directional elements in the Lewisian Complex. The evolutionary aspects become clarified if the system of Lewisian structures comparable with the dislocations of the Nagssugtoqidian regime are regarded as relatively early elements in an integral system related to, if not initiated by, movements along steep dislocations associated with a major continuation of the Ketilidian Belt which eventually dominated the part of the craton which is now the Lewisian.

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A psychological analysis of observation in geology

ANALYTICAL methods of experimental psychology applied to observations of geological data reveal that what geologists perceive in, and remember of, rocks is not necessarily the same as what is actually there. For example, when observing folds, professionals give more attention to antiforms than synforms and tend to remember cleavage fans as they ought to be rather than as they are.

Geology is a science which relies very heavily, at the stage of data collection, on the sense of sight. Experimental methods, which allow an assessment of common biases in perceiving and memorising can show where presented evidence could be distorted. Standardised observation tests are also valuable for training because they provide accurate feedback. This is not always obtained easily during actual geological work because 'the solution' is itself what is sought—it is not known beforehand.

To avoid continually concentrating on biases in observation the investigation reported here was also directed towards finding evidence of perceptual attributes so that these, if present, can be used more widely.

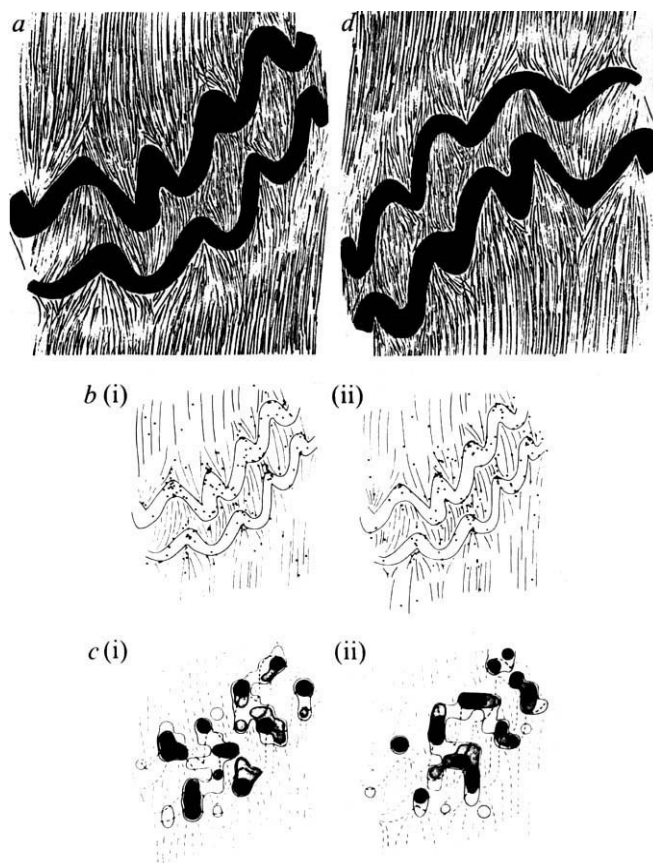
For standardisation I constructed stimulus items of controlled content and complexity. In order, however, for the geologists to construe the items as symbolic of rocks, they were not drawn too schematically.

To examine possible location and shape biases in the visual assessment of folds, 22 geologists and 22 non-geologists were individually shown Fig. 1a. They were instructed to look at the picture and, at 10 random moments chosen by the experimenter, to indicate, by pointing, where they were looking. The indicated points were then recorded on an outline copy of the original. Figure 1b shows the synopsis of plots from both groups of subjects and Fig. 1c shows the data contoured.

Several points emerge clearly from this simple experiment. First there are far more plots in antiforms than synforms ($0.001 > P > 0.0001$), more in hinges than limbs and more in the black buckled layers than in the sandwiched, less competent layers (an average of 50 compared with 36). There are also more plots in the upper than in the lower half of the scene (191 plots compared with 133, central layer data neglected). Non-geologists differed markedly from geologists in having more plots in the upper halves of layers than in the lower halves (irrespective of whether antiforms or synforms are considered). Within the upper layer the difference in plots for non-geologists is at the 0.1% level and for the lower layer $P = 0.05$; (for geologists $P > 0.25$ for both). This accords with the introspections of the control group—many saying that they tended preferentially to scan the upper boundaries. Non-geologists also gave less attention to the (what is to a geologist) unusual fold at the left in the upper thin layer (see Fig. 1b and c).

There was no tendency in either group for there to be more concentration on the outer arcs of folds rather than the inner arcs ($P > 0.25$ for both groups).

Fig. 1 Geologists tend to describe (a) as comparing fairly tight angular upright folds and (d) as comprising well rounded fairly open folds. Yet (d) is merely (a) rotated through 180°. b, Plots of location-of-gaze (i, 22 geologists; ii, 22 non-geologists); c, contoured (at 1, 1.5, 2 and > 2% intervals) data from (b) (i, 22 geologists; ii, 22 non-geologists).



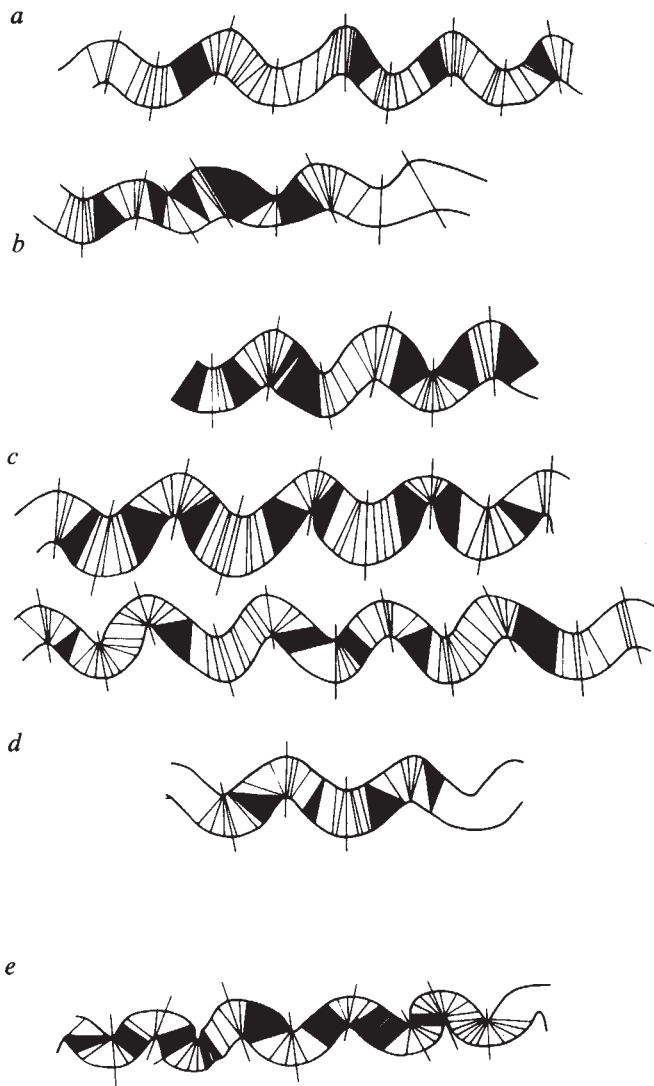


Fig. 2 Isogons (lines connecting points at which interface dips are equal) drawn on folds in which the amplitude and arc length of deflection of the inner and outer arcs is unequal. *a-e*, Five randomly selected portions of fold trains of buckled sandy layers in silt (Ordovician, Hommelvik-Hell region, Norway). Black areas, 'zones of no plots' where no isogons are possible.

It seems that irrespective of the speciality of the geologist the buckled thinner layers tend to be perceived as antiform 'chunks' joined at synform hinges, not the converse (compare Fig. 1*a* with Fig. 1*d*).

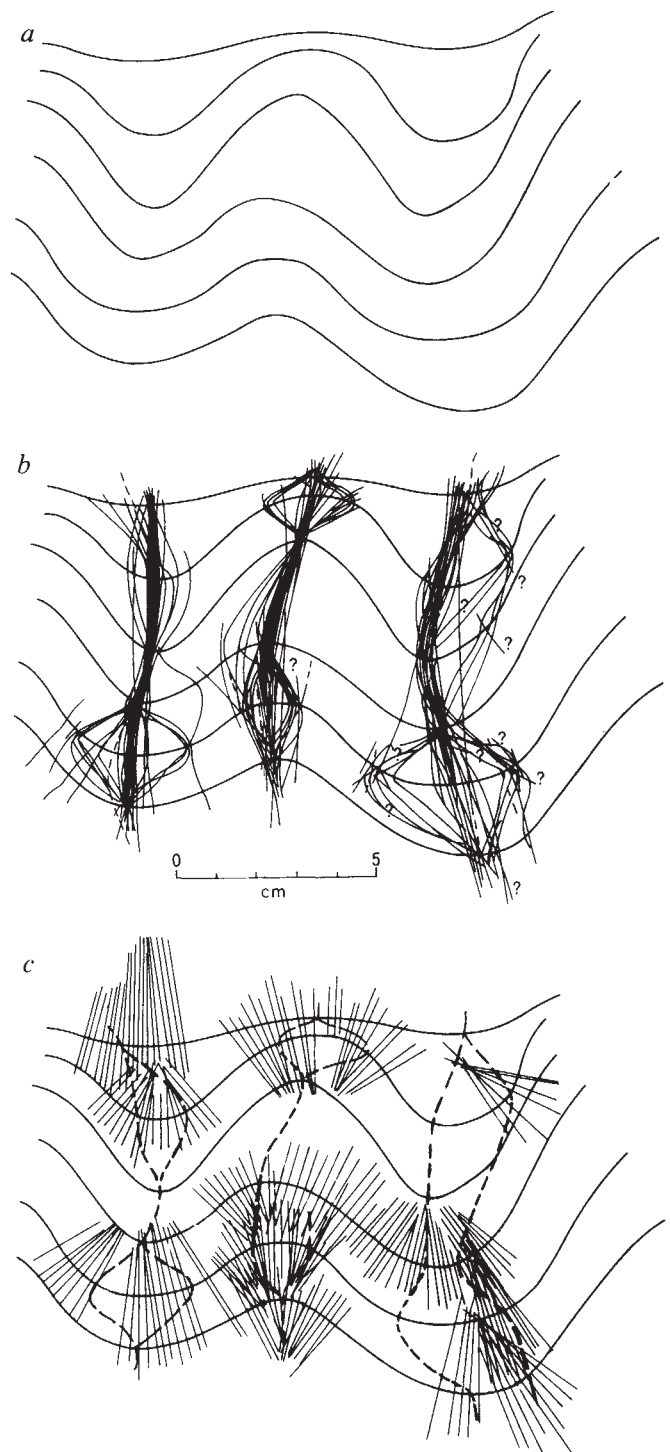
The preference towards selecting antiforms may be reflected neurologically in a biasing at the level of complex cells in the occipital cortex where there is sensitivity to both orientation and spatial location. Alternatively, one could conceive of the preference for antiforms as resulting from a tendency to look at the upper halves of many objects in everyday life in order to extract the maximum amount of most useful information in the shortest time. That strategy may be transferred to scientific work—in this case to the observation of fold pairs—leading to differential visual scrutiny. The experiment indicates that when geologists report, say, that "the most common F_2 folds" in an area are of a certain type, they may, to a large extent, be reporting what are really the most common antiformal folds.

That is a serious bias because antiforms and synforms may differ systematically both in geometry and interior

detail when, for instance, a folded layer is graded or current bedded. It is, therefore, important to remember that synforms are just as important as antiforms and should not be neglected in visual work.

The concentration of visual attention on hinge zones of folds at the expense of limbs, may be one of the reasons why one limitation in Ramsay's classification of folds¹ has been neglected. The use of isogons in the analysis of fold

Fig. 3 Geologists were requested to construct axial surfaces through the folded layers in (*a*) (taken from the Dalradian Ballachulish Limestone on Eilean Munde in Loch Leven, Argyllshire); *b*, combined data from all subjects; *c*, my interpretation (most of the construction lines used to locate hinge points have been left on; axial surfaces dashed).



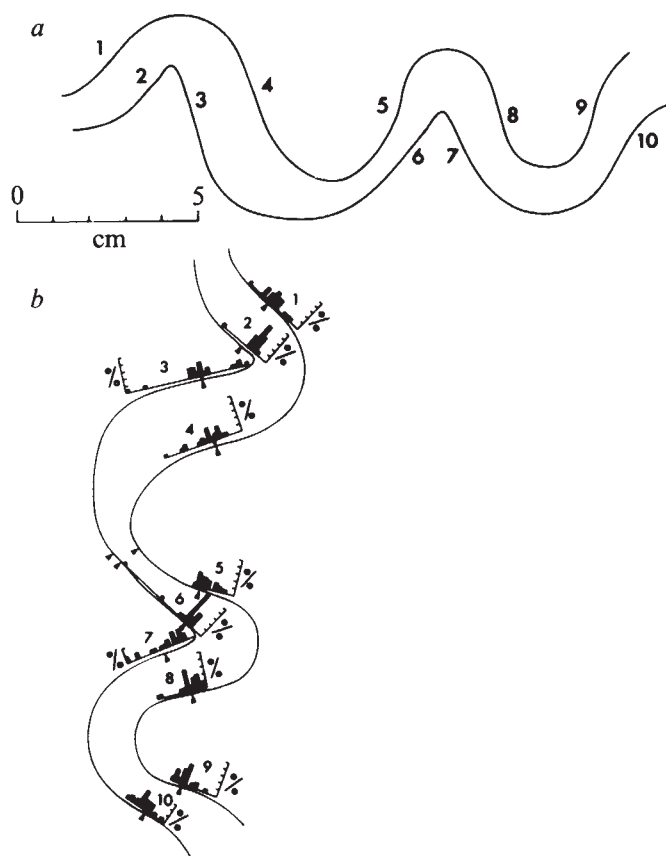


Fig. 4 Geologists were requested to locate inflection points on the limbs of the folds shown above. Below are microhistograms (ordinate was 10% divisions) of the locations with data combined from 22 structural geologists. Arrows, actual inflection points in the original example.

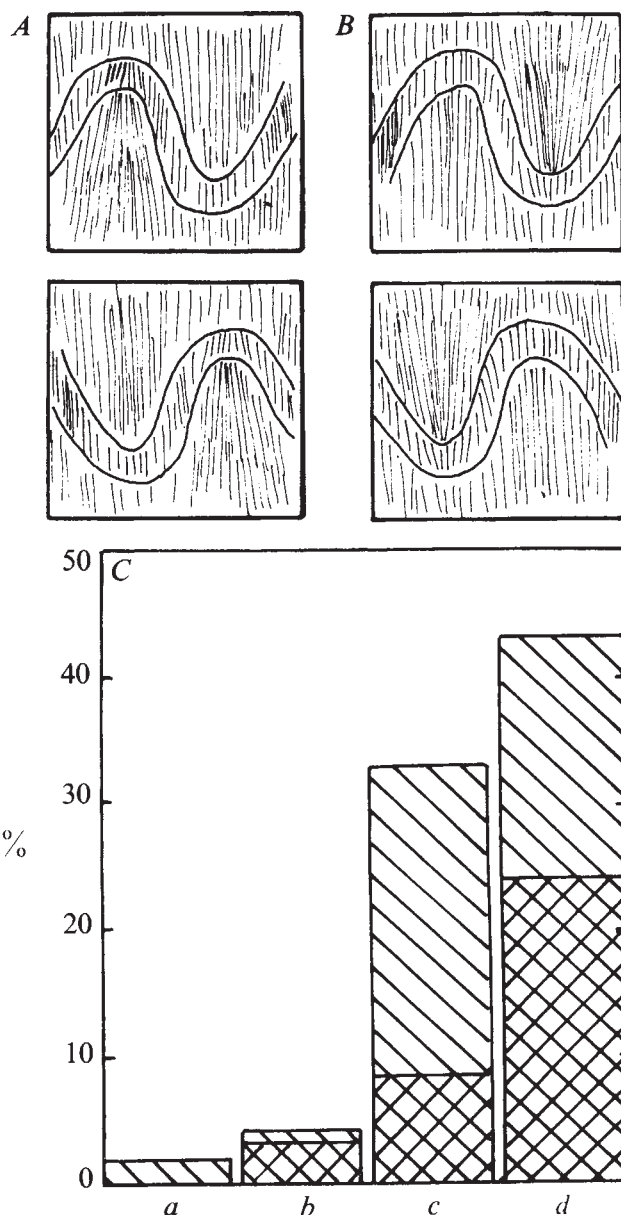
geometry can only be fully applied if the limb dip at the inflection points of the inner and outer arcs is identical. To the unaided eye, they seem to be closely the same in strongly flattened folds but at lower shortenings there is usually a portion of the limb which clearly cannot be analysed because of the differential dip of the two layer surfaces. In Fig. 2 the black areas are those in which isogons cannot be constructed and orthogonal thickness cannot be measured. Differences in amplitude and arc length between inner and outer arc and differences in shape between antiforms and synforms are inevitable in natural folds because the resistance on one side of a layer will never be exactly the same as the resistance to growth in the opposite half space. The differences would be even greater if the layer were graded.

Thirty structural geologists were requested to construct axial surfaces through the folded laminations shown in Fig. 3a; a synopsis of the constructions from all subjects is shown in Fig. 3b, with my interpretation constructed on an enlargement ($\times 3$) (Fig. 3c). Apart from the expected variability in the constructions, 60% of the subjects tended, in this 'open ended' task, to avoid any construction of bifurcating axial surfaces. Only 12.5% drew all three surfaces as bifurcating in at least some places. This is an omission in analysis which could lead to an inordinately low impression of the prevalence of conjugate and box-fold geometries in naturally deformed rocks. This neglect of multiple-hinged folds is also reflected by the fact that most geologists do not expect conjugate folds to be present on a mappable scale.

Twenty-two structural geologists were requested to locate inflection points on the folded layer interfaces shown

in Fig. 4. In these moderate and high limb dip-folds at least, the inflection points tended to be located too high up on the limb by 17 of the 22 subjects, irrespective of the dip direction of the limb. The overall mean ratio of up-dip to down-dip locations is 3.25:1 ($0.001 > P > 0.0001$). Were such folds being so subdivided for Fourier analysis, it would lead to a spurious increase in synform amplitude and wavelength and a complementary decrease in those parameters in the antiforms. To compensate for the bias the

Fig. 5 A divergent cleavage, when presented in a buckled layer, is reproduced more frequently when presented in the antiform (A), than when in the synform, (B), irrespective of whether positioned on the left or the right in the visual field. The object was a slide stimulus projected so as to measure about $2\text{ m} \times 2\text{ m}$. Subjects were requested to reproduce the item by freehand drawing immediately after stimulus offset, and (C) shows the percentage of subjects who remembered the presence of the divergent cleavage in the folded layer (cross-hatching, when the cleavage occurred in the antiforms; criss-crossing, in the synforms). Subjects were tested in groups: a, inexperienced and pre-university; b, 85 subjects who were beginning geology, were university undergraduates or were 'O' or 'A' level pupils; c, 77 subjects with 1–2 years' university experience; d, 6 subjects with between 2 and 36 years' experience. (Note that in this case experience is correlated with age.)



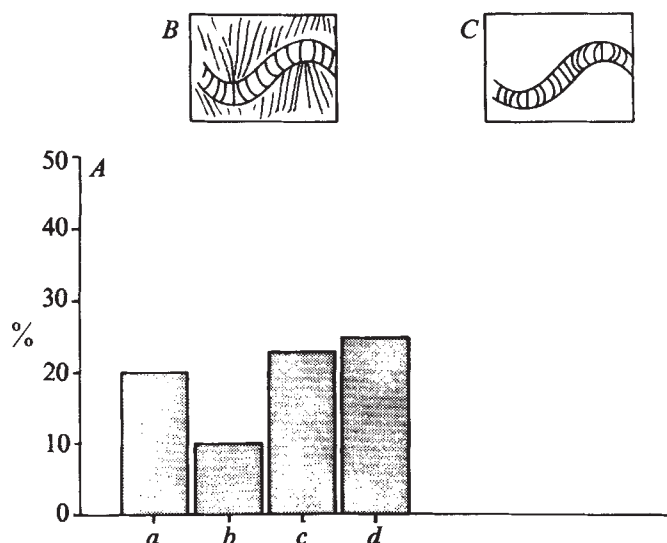


Fig. 6 A, Percentage of cleavage reproductions that were shown as symmetrical from synform to antiform by subjects requested to reproduce the asymmetrical cleavage patterns shown in (B). a, 53 inexperienced subjects; b, 31 'O' level pupils; c, 54 subjects with 1–2 years' experience; d, 41 subjects with more than 2 years' experience. C, Common reproduction of (B).

inflection points could be located twice with the fold train rotated through 180° for the second analysis. In those cases where the two points are not identically placed a mid-point between them could be used.

Accuracy of recall from visual short term memory is necessary in observation work; for instance, in making field notebook drawings. To examine such recall, 300 subjects with experience of geology ranging from 2 weeks up to 36 years, in groups ranging from 6 to 50 in number, were shown a randomised sequence of 10 slides which represented varied cleavage fan geometries in folds of single layers. The subjects were required immediately to draw each of the cleavages from memory after viewing the slide stimulus

for either 7 s or 10 s, depending on the slide. This relatively short viewing time was given because of the limited detail in each slide.

The first conclusion from this experiment is that reproductions of antiform cleavage fans are much better than those for synformal cleavage fans (Fig. 5). The bias towards better antiform reproduction does not decrease with experience (a divergent cleavage—in the buckled more competent layer—was used here as a stimulus item because it was never, in any of my previous experiments, wrongly reproduced as a guess by any subject when the stimulus item did not contain it). Cleavage fans that were asymmetrical from antiform to synform (Fig. 6), as could occur, for instance, in a folded, graded layer, tended to be reproduced, if at all, as symmetrical from antiform to synform by about 20% of subjects in each experience group.

In some groups the first slide presented had curved cleavage traces and the third was similar to it in many respects, except for the fact that the cleavage traces were straight. The percentage of subjects who misremembered the cleavage in the third slide as curved (that is, the percentage who transposed detail from the first to the third item) did decrease as a function of experience (Fig. 7). On the other hand, cleavage traces which were convergent in the matrix inside the inner arc of the buckled layer were more often misremembered by professional structural geologists as divergent. This is the way matrix cleavage fans are usually represented in text books of structural geology and also, presumably, in the long term memories of the structural geologists.

It was found that the way the object on the screen was construed by subjects could vary as a function of bias introduced by the experimenter and that this could influence the scanning and search strategies used. For instance, a palaeontologist who came into a laboratory during the display of one of the sequences (and who had therefore missed the initial instructions) thought that the so-called folds and cleavages were worm-tubes and disrupted laminations. On the basis of introspection he detected no evidence of antiform preference in himself. Rather than looking mainly at the antiforms and, by redundancy, assuming the synform to be symmetrical, he instead scanned along the 'tube' and therefore recognised the differences between the antiforms and the synforms.

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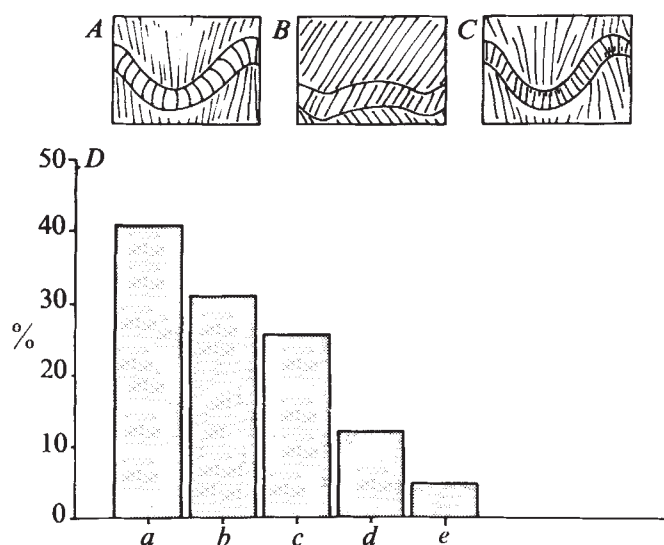


Fig. 7 Subjects were shown A, B and C, and asked to reproduce them; D, percentage of subjects who incorrectly reproduced the layer cleavage in (C) as curved: a, 44 subjects; b, 32 'O' Level pupils; c, 40 'A' level and first year university students; d, 51 subjects with more than 1–2 years' experience; e, 40 subjects with more than 2 years' experience.

Exercise and human sleep

It has been proposed that slow wave sleep (SWS) in humans is associated with somatic restitution¹, and findings of increased plasma somatotrophin during SWS², and of the greater amounts of SWS following exercise^{3,4} support this proposal. Others have, however, failed to show any exercise effects of sleep^{5,6}. Exercise has been found to increase sleep somatotrophin levels, but not SWS⁷. These contradictory findings may in part be attributed to the large interstudy variations of administered exercise and fitness of subjects (in no study has exercise been standardised against individual work capacity); the time of day of exercise; the need for further qualification of SWS, defined⁸ here as sleep stages 3 and 4, respectively containing 20–50% by time of delta activity, and more than 50% delta activity. Up to 20% delta activity is included in the non-SWS stage 2. We have investigated the effects of the time

Table 1 Group means (%) for sleep stages

	Baseline	Morning exercise		Afternoon exercise	
		Recovery night	Carryover night	Recovery night	Carryover night
Whole night					
Stages 0 + 1	5.2	6.2	5.7	5.4	4.8
2ii	9.9	8.8	8.9	11.7	9.6
3	9.5	9.7	10.8	10.8	8.5
4	18.2	19.1	17.2	17.2	16.8
3 + 4	27.7	28.8	27.9	27.9	25.3
2ii + 3 + 4	37.6	37.6	36.8	39.6	34.9
REM	23.5	25.2	24.1	23.7	24.4
First half					
Stages 0 + 1	2.8	3.2	3.8	2.7	2.6
2ii	11.6	8.0†	10.0	14.6	12.2
3	13.2	11.5	13.6	16.2†	11.0
4	31.6	35.3	26.1	29.3	30.8
3 + 4	45.1	46.7	39.7	45.3	41.9
2ii + 3 + 4	56.7	54.7	49.7*	59.9	54.1
REM	13.7	15.9	16.3	13.2	14.5
Second half					
Stages 0 + 1	7.8	9.2	7.6	8.2	7.1
2ii	8.2	9.5	8.1	8.8	7.1
3	5.7	8.0	7.9	5.3	6.1
4	4.9	2.8	8.2	5.1	2.8
3 + 4	10.4	10.9	16.1	10.8	8.7
2ii + 3 + 4	18.6	20.4	24.3	19.6	15.8
REM	33.3	34.4	31.8	34.7	34.4

*Significant at 0.05 level (two-tailed).

†Significant at 0.01 level (two-tailed).

of day of individually standardised amounts of exercise on subsequent sleep in which stage 2 was subdivided further between 10% and 20% delta activity (2ii).

Eight healthy male subjects (18–22 yr), of average build and fitness, were assessed for individual maximum aerobic power during steady-state exercise on a bicycle ergometer by the Nomogram method⁹. From the maximum work capacity calculated, each subject was prescribed a workload 45% of this capacity. It was further established that this load could be maintained without undue stress for 85 min, with a 15 min break at the half way point. In the main study the exercise was performed by each subject on two separate days, once in the morning, and once in the afternoon. Electroencephalograms were obtained throughout the sleeping period for each exercise night, and for a following 'carryover' night. There were initial adaptation nights and two baseline nights. Subjects were measured in pairs with one performing morning exercise and the other evening exercise on any one day. Diet, naps, extraneous exercise and alcohol intake were controlled. Sleep records were independently scored into the recognised sleep stages⁸ and stage 2ii. Group data were compiled for the sleep variables shown in Tables 1 and 2. Related *t* tests were used to compare the data for each of the experimental nights with the average baseline values.

Tables 1 and 2 show that there are few significant findings. Whole-night percentages of each sleep stage and parameter,

including 2ii, during all experimental nights remained within baseline limits. During the first half of the afternoon exercise night, and particularly before the first rapid eye movement (REM) period, stage 3 showed a significant increase. Although stages 4 and 2ii before the first REM period tended to show increases, they did not individually reach significance. On this night three subjects missed their normally expected first REM period, producing an unusually long delay to a first REM period and enabling more SWS to occur before the first REM period. These three REM period delays seem to be mainly responsible for the longer, but non-significant, REM latency. There are no significant effects for any parameter during the afternoon exercise carryover night. Stage 2ii shows two related significant decreases on the morning exercise night, one before the first REM period and the other for the first half of the night. Any possible loss of delta activity here, however, may have been made up in the slight increase of stage 4, the more substantial delta activity sleep, during the first half of the night. On the morning exercise carryover night, summated delta activity stages 2ii + 3 + 4 showed a significant decrease during the first half of the night, which seems to be reciprocated by a significant increase during the second half. This seems to be a real temporal displacement, rather than an artefact of the division line of sleep into halves. The reason for this is not known.

Our findings seem to show a time of day effect of exercise on

Table 2 Group means for REM parameters

	Baseline	Morning exercise		Afternoon exercise	
Time (min) before first REM period		Recovery night	Carryover night	Recovery night	Carryover night
Stages 0 + 1	1.0	0.7	1.4	2.1	1.7
2ii	10.6	5.4*	7.8	16.6	9.3
3	14.4	10.7	12.5	19.6*	11.6
4	47.9	50.2	42.0	56.2	40.0
3 + 4	62.1	60.9	54.5	75.7	51.6
2ii + 3 + 4	72.7	66.3	62.3	92.3*	60.9
REM latency	91.9	79.4	79.4	112.9	89.5
First REM period length	14.4	11.9	9.0	15.8	10.9
REM periodicity					
First period	88.4	87.9	77.2	77.1	81.7
Mean of first and second periods	92.3	92.0	90.4	89.9	89.6

*Significant at 0.05 level (two-tailed).

recovery sleep. The main SWS delta activity increase was for stage 3 during the first half of the night following afternoon exercise, and this seems to be partially counterbalanced by a non-significant decrease during the second half of the night, resulting in no significant overall changes. If SWS reflects enhanced protein synthesis and body restitution¹, then in view of the high workload imposed, more substantial changes in SWS might have been expected. It must be concluded that if a heavy, but tolerable, workload is imposed early in the day then ensuing wakefulness is sufficient for recovery. If this exercise is given later in the day, however, then ensuing wakefulness may not be adequate for recovery, and some of the recovery process may intrude into the earlier part of sleep and be reflected in the sleep EEG.

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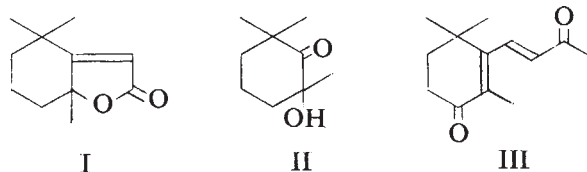
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Dihydroactinidiolide in the supracaudal scent gland secretion of the red fox

IN spite of growing interest in the function of skin glands in mammalian chemical communication^{1,2}, little is known of either the chemical nature or the behavioural significance of the secretion of the supracaudal (tail) scent gland of the red fox (*Vulpes vulpes*). A recent paper³ reports observations concerning the histology and histochemistry of this gland, including findings of unusual fluorescent sebum constituents. In this paper, preliminary gas chromatographic-mass spectrometric data are presented indicating the presence of dihydroactinidiolide (I) and related compounds in this secretion and the significance of this finding is discussed.



Particles of waxy yellow-brown stale secretion were separated manually from the hair and skin surface of the supracaudal gland region of eleven male red fox (taken wild, February 14, 1974–April 19, 1974, Wales), bulked (67 mg) and distilled (at 170 °C, air bath temperature, 0.02 mmHg). The distillate was examined by combined gas chromatography-mass spectrometry (AEI MS30 mass spectrometer (24 eV) with a Pye-Unicam 104 gas chromatograph fitted with a 2 m × 2 mm internal diameter glass column of 2% SE-33 on 80–100 mesh Gas Chrom Q; column temperature 116 °C to 280 °C at 5 °C min⁻¹; injector temperature 170 °C; separator temperature 200 °C) following treatment with diazomethane.

A number of volatile components exhibiting mass spectra suggesting monoterpenes or related compounds were observed

in advance of the range of saturated carboxylic acids (starting at C-14, observed as methyl esters) and related compounds expected in a distillate of skin lipids. These more volatile compounds included substances possessing the following observed mass spectra (five most intense ions, plus other significant ions) indicating dihydroactinidiolide (I)⁴ *m/e* 180 (*M*⁺, 22), 165 (7), 152 (11), 137 (30), 111 (100), 109 (30), 43 (25); 6-hydroxy-2,2,6-trimethylcyclohexanone (II)⁵ *m/e* 156 (*M*⁺, 6), 128 (31), 110 (35), 95 (39), 71 (100), 58 (34), 43 (36); and *trans*-4-keto-β-ionone (III)⁶ *m/e* 206 (*M*⁺, 47), 191 (15), 177 (6), 164 (38), 163 (91), 121 (53), 43 (100), 41 (38). Other major volatile terpenoid constituents of the secretion distillate are under investigation.

The identity of dihydroactinidiolide was confirmed by gas chromatographic coinjection with authentic material, m.p. 42–43 °C (retention time 4.1 min, 1.5 m × 2 mm internal diameter glass column of 5% Dexsil 300-GC on 80–100 mesh Chromosorb W, AW; column temperature 172 °C, isothermal). A peak of the same retention time was also noted when a thin-layer chromatography fraction (acetone pre-eluted silica gel G (Merck type 60), *R_f* 0.06 to 0.2 with diethyl ether-petroleum spirit (60–80 °C), 30/70, v/v) of a methanol-chloroform extract of stale secretion was examined by gas chromatography (dihydroactinidiolide, *R_f* 0.08). The level of dihydroactinidiolide in this sample of stale secretion was < 50 p.p.m.

This is the first report of volatile terpenes in an external mammalian glandular secretion, although it was expected that such compounds might be involved in mammalian communication following the elucidation of the olfactory response of certain Felidae to *cis-trans*-nepetalactone, a major constituent of the essential oil of the catnip plant, *Nepeta cataria*⁷. The early description of the supracaudal gland as the 'violet gland' on account of its odour also suggested such a finding. A number of terpenoid constituents of the plant *Actinidia polygama*, including dihydroactinidiolide, are also reported to have behavioural effects on certain Felidae⁸.

The presence of photolabile fluorescent compounds has been reported in the red fox supracaudal gland sebum, and fluorescence and ultraviolet spectra have been described³. Field desorption mass spectrometry of the major non-polar fluorescent fraction (Varian CH5D, source temperature 80 °C, emitter current 8 mA) revealed major ions at *m/e* 524, 525, 550 and 552 (no ions in range *m/e* 554–1,200) indicating the presence of compounds in the carotenoid mass range. If carotenoids are present, their occurrence could be related to the presence of volatile terpenes in the secretion⁹.

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Ethylene in soil

THE origin of ethylene in the soil atmosphere is of interest because the concentrations at which it sometimes occurs can restrict plant growth by inhibiting root extension in cereals¹ and root nodulation in legumes²; it has also been suggested that it aids plants by inhibiting pathogenic fungi³. Our earlier observations showed that ethylene could be produced by a soil fungus, *Mucor hiemalis*, and two soil yeasts, which have now been identified as *Candida vartiovaarai* and *Trichosporon cutaneum*, when glucose and methionine were available to them⁴. Subsequent investigations in pure culture and in soil have attempted to elucidate mechanisms by which these organisms could synthesise the gas⁵⁻⁷. Other investigators^{8,9} have proposed that anaerobic spore-forming bacteria rather than fungi are largely responsible for ethylene formation in soil as: (1) heat does not prevent the formation of the gas in soil, (2) anaerobiosis favours its formation and (3) the gas is fungistatic. Further experiments have therefore been undertaken in an attempt to assess if these latter observations cast doubt on our conclusions; the results are reported here.

Using previously described techniques⁸ we have confirmed earlier reports that heat⁸ and even autoclaving⁹ can stimulate the production of ethylene by soils (Table 1). The effect of heat at 100 °C on the release of ethylene from pure cultures of *M. hiemalis* was therefore investigated. No ethylene was produced by heating the culture medium alone but, although the treatment killed the fungus, the evolution of the gas from the treated culture was greatly enhanced compared with that of cultures kept at 25 °C (Fig. 1). Some observations in our earlier work suggest that a possible explanation for this is that *M. hiemalis* forms an intermediate substance in the pathway to ethylene which passes into the extracellular solution and breaks down non-enzymically, a process promoted by heat at 100 °C (ref. 6). Another possible explanation is that heat decreases the growth rate and in the range of growth rates 0.08 h⁻¹ to 0.02 h⁻¹, there

Fig. 1 Effect of heat (100 °C per 30 min) on ethylene production by pure cultures of *Mucor hiemalis*. The fungus was grown in glucose-methionine medium⁵ (100 ml) in flasks (capacity 325 ml) for 48 h to produce 1.5 mg dry weight ml⁻¹. The cultures were then heated and the flasks sealed 30 min after the treatment. The heated medium alone produced no ethylene. ●, Heated; ○, unheated.

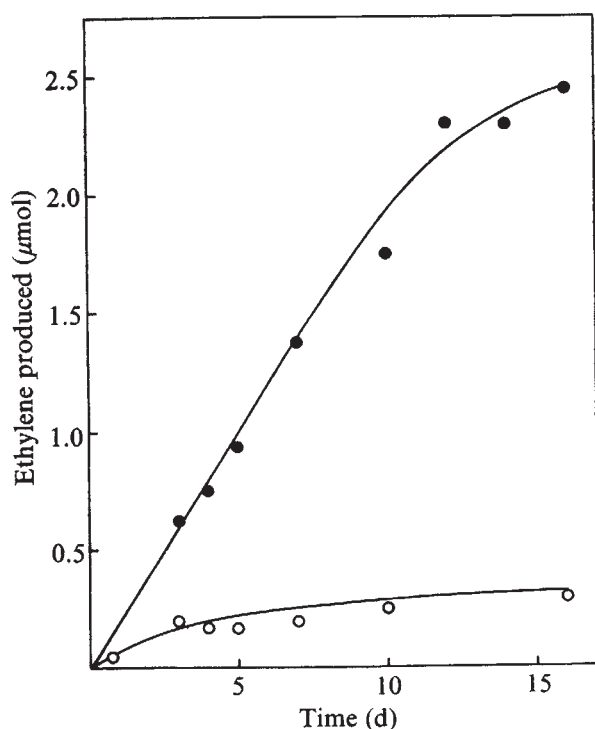


Table 1 Effect of heat treatment on ethylene production by two soils

Treatment	Ethylene nmol per 100 g soil per 6 d	
	Chalk loam (Gore series) 6.6% organic matter	Basaltic loam (Woodburn series) 9.8% organic matter
Boiling (100 °C per 10 min)	8.0	23.4
Autoclaving (121 °C per 60 min)	3.4	7.2
Control (unheated)	0.7	22.9

Soils (5 g) were wetted and incubated in glass vials (14 ml)*.

is an exponential increase in ethylene production⁷; furthermore the subsequent cell lysis also increases the production of the gas⁵.

The production of ethylene after heating soil is therefore consistent with a fungal origin. Moreover we have obtained evidence that although ethylene is normally produced in anaerobic conditions¹⁰, the importance of these conditions seems to lie in the provision of substrates, which we have recently found can be provided by crop residues. When suitable substrates, glucose and methionine, are provided the production of ethylene by *M. hiemalis* and by soil is stimulated by oxygen⁵. Furthermore anaerobiosis can reduce fungal growth rate which increases ethylene production^{5,7} and inhibits ethylene breakdown by soil microorganisms¹¹. Waterlogging, of which anaerobiosis is a consequence, inhibits gaseous diffusion and retains ethylene in the soil atmosphere.



Fig. 2 Colonisation of buried straw by a mixed fungal population (identified as *Mucor* sp. and *Fusarium* sp.) in the presence of ethylene; similar fungal development has been observed in the presence of 1,000 p.p.m. ethylene. Saprophytic fungi are the most likely causative agents of ethylene production from organic residues in soil.

If the inhibition of growth by ethylene was a normal characteristic of fungi, they could not be considered as the main producers of the gas in soil. But whereas the pathogenic fungus *Sclerotium rolfsii* is very sensitive to ethylene³, five other fungal species investigated in this and three other laboratories were found to be relatively unaffected by considerably higher con-

centrations than those found in soil (Table 2). Figure 2 shows the extensive mycelial development which can occur in the presence of high concentrations of the gas and while it might be postulated that ethylene would be fungistatic only in nutrient-limited conditions, we have found little evidence for any soil microbial activity in these conditions.

Table 2 Effect of ethylene on fungal growth

Fungus	Concentration of ethylene (p.p.m.)	Maximum inhibition of growth or germination (%)	
		<i>In vivo</i>	<i>In vitro</i>
<i>Sclerotium rolfsii</i> ³	1	100*	—
<i>Fusarium oxysporum</i> ¹²	2,000	16†	57
<i>Gloeosporium album</i> ¹³	2,000	33†	19
<i>Sclerotinia fructigena</i> ¹⁴	100	22†	12
<i>Mucor hiemalis</i>	1,000	0‡	0§
<i>Aspergillus clavatus</i>	1,000	0‡	—

—, No information.

*Grown in soil.

†On fruit tissue.

‡On decaying barley tissue in soil.

§In 1/100 strength tryptone soya broth.

In our earlier work⁴ bacterial isolates from soil produced little or no ethylene whereas fungal isolates produced large amounts. This observation has now been confirmed and in addition studies in pure culture with the ubiquitous soil anaerobe, *Clostridium pasteurianum*, showed negligible production of ethylene. Moreover the only report in the literature on the production of ethylene by bacteria in pure culture relates to *Pseudomonas solanacearum*¹⁵; in contrast, cultures of over 70 fungi have been shown to produce ethylene¹⁶. No information now available therefore is inconsistent with saprophytic fungi being responsible for the production of ethylene and in the soils we have studied *M. hiemalis* seems to be a major contributor. This does not prove that anaerobic bacteria do not produce ethylene but indicates that their contribution if any is very minor.

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Cytogenetic effects of plutonium-239 in male mice

INCREASING quantities of plutonium-239 are processed in the nuclear power industry. The likely magnitude of the associated genetic risk is still uncertain but thought to be less than the risk of cancer induction¹. Green *et al.*² have recently shown, however, that plutonium reaching the testis after intravenous injection of ²³⁹Pu citrate into CBA mice concentrates in the interstitial tissue, outside the seminiferous tubules. They calculated that the average dose rate to spermatogonial stem cells, in which genetic damage can accumulate, was about 2–2.5 times

that to the whole testis. In these circumstances, the genetically significant dose is higher than the average tissue dose, which is that normally used for protection purposes³. To obtain more direct evidence on genetic damage from gonadal plutonium, we are studying the induction of chromosome aberrations in germ cells of male mice after protracted exposure to α particles from ²³⁹Pu. The results of the first experiment, described here, suggest that α radiation has the expected high relative biological effectiveness (RBE)³ for induction of chromosome damage and that a distribution factor of the magnitude proposed may indeed be operating as well.

Twelve 10-week-old (C3H/HeH $\times$ 101/H δ) F₁ male mice were injected intravenously with 10 μ Ci kg⁻¹ ²³⁹Pu in 1% trisodium citrate solution (about 0.3 μ Ci per mouse). Three litter-mate controls were injected with similar quantities of the citrate solution alone. Cages of four treated and one control were kept for 6, 12 and 18 weeks. They were then killed and weighed, as were the testes. Air-dried chromosome preparations⁴ were made from cellular suspensions of each right testis, and the left was used for radiochemical assay of plutonium. From each testicular preparation, 100 spermatocytes at diakinesis-metaphase I were scored for chromosome anomalies. Additional procedures on the 18-week batch only were sperm counts from the caput epididymis⁵; examination of 500 spermatozoa from the cauda epididymis for head abnormalities, after staining with eosin Y and making air-dried smears⁶. All slides were coded.

The fraction of injected plutonium retained in the left testis changed very little over the period of the experiment (Table 1); therefore radiation dose was taken to be the product of the assay-derived dose rate (≈ 0.0002 rad min⁻¹) and time. Testis weight declined to 58% of controls after 18 weeks (Table 2). At this time, the mean epididymal sperm count was only 51% of the control value, showing that much of the testis weight loss was the result of germ cell death. The mean frequency of sperm-head abnormalities was 9.0%, compared with 4.6% in controls, but this difference mainly stemmed from one male with 17.2% abnormalities. The causes of these abnormalities are not yet known, but they are thought to indicate genetic damage⁶.

In the experimental series, frequencies of autosomal and X–Y chromosome univalents did not differ significantly from control values of $6.0 \pm 1.4\%$ and $12.0 \pm 1.9\%$, respectively. The frequencies of spermatocytes with fragments (Table 2) showed a non-significant increase in the irradiated series; 87% of these fragments were diagnosed as double and thought to be the result of isochromatid breaks. Whereas affected control cells had one fragment each, six cells in the irradiated series had two fragments and three cells had three. Thus the overall fragment frequency after treatment was $3.4 \pm 0.7\%$, compared with the control frequency of $1.3 \pm 0.7\%$ ($P = 0.07$ on one-sided test).

The increase in the frequency of spermatocytes with quadrivalent configurations (Table 2) in the treated series is clearly significant. Most of these were typical rings and chains of four arising from chromosomal reciprocal translocation in spermatogonia, as previously found after X, γ and neutron irradiation of this cell type⁷. A few, however, (especially at the 18-week interval) seemed to resemble more closely the type of quadrivalent previously seen by two of us after X irradiation of

Table 1 Results of radiochemical analysis of left testes of hybrid mice after injection of ²³⁹Pu, with determinations of average radiation dose

Weeks of exposure	No. of mice	²³⁹ Pu in left testis ($\pm$ s.e.) % Injected activity	pCi	Dose* (rad)
0	3	—	—	0
6	4	0.042 ± 0.002	130 ± 9	14
12	4	0.048 ± 0.002	143 ± 13	30
18	4	0.040 ± 0.004	134 ± 28	44

*For calculations of dose, the average of the control and the mean experimental left testis mass for the batch concerned was used, and the mean energy of a single α particle from ²³⁹Pu was taken as 5.15 MeV (ref. 16).

Table 2 Mean testis mass and chromosome aberrations at metaphase I at different times after injection of ^{239}Pu (percentages in brackets)

Weeks of exposure	Testis mass (mg $\pm$ s.e.)	Spermatocytes scored	Number with		Reciprocal translocations		
			Fragments	Quadrivalents*	Rings	Chains	Totals
0	125 $\pm$ 2	300	4 (1.3)	1 (0.3)	0	0	0 (0.0)
6	91 $\pm$ 6	400	8 (2.0)	4 (1.0)	3	1	4 (1.0)
12	88 $\pm$ 2	400	9 (2.2)	16 (4.0)	15†	4	19 (4.8)
18	73 $\pm$ 8	400	12 (3.0)	9 (2.3)	2	3	5 (1.3)

*Resulting from reciprocal translocations arising in spermatogonia (see next columns) or chromatid interchanges in spermatocytes.

†Three cells each had two rings of four.

oocytes in female mice⁸, they were therefore thought to be the result of chromatid interchange in spermatocytes during meiotic prophase.

The yield of quadrivalents was reduced at the longest exposure period, the effect being most marked in the translocation group. A similar decline in translocation frequency has been reported after high acute exposures to X rays^{9,10} or fission neutrons¹¹, but not after protracted exposures to the latter. The same phenomenon occurs with specific locus mutation frequencies after acute exposures¹² and is thought to be connected with the preferential killing of the most mutationally sensitive part of the spermatogonial population by high radiation doses. It is possible that a similar explanation could account for the decrease in the present experiment. Consequently, the low 18-week values have been excluded when determining rates of induction.

The data on reciprocal translocation induction can be used to obtain a rough estimate of the relative effectiveness of the α particles. As these translocations would be expected to arise in spermatogonia rather than spermatocytes (where the chromatid interchange type would be found), the time taken for the germ cells to pass through meiosis to metaphase I (13 d)¹³ should be subtracted from the actual exposure period, to give the period of spermatogonial exposure. When these amended doses of 10 and 25 rad are used and linearity of response is assumed the rates of induction of reciprocal translocations at the 6-week and 12-week exposure periods are 1.0×10^{-3} and 1.9×10^{-3} rad⁻¹, respectively, with a mean of 1.45×10^{-3} rad⁻¹ of the average testis dose. In previous experiments with the same hybrid male mice, a 600 rad spermatogonial dose of γ rays delivered at 0.02 rad min⁻¹ (ref. 14) induced translocations at the rate of 2.3×10^{-5} rad⁻¹, whereas a 62-rad dose of fission neutrons, delivered at 0.0005–0.0008 rad min⁻¹ (ref. 11) induced them at a rate of 0.53×10^{-3} rad⁻¹. These rates are lower than that estimated for ^{239}Pu α particles by factors of 63 and 2.7, respectively. Thus these results are in good agreement with the assumption that α particles have a similar RBE for translocation induction to that of fission neutrons (previously reported as about 23 for chronic exposures)¹⁵ and that the α -particle dose to spermatogonia is about 2.5 times the mean dose to the whole testis². There are, however, wide error limits to some of these values as well as uncertainty on whether more protracted γ irradiation would have the same effect as the 0.02 rad min⁻¹ dose quoted above. Therefore further work is in progress, from which we hope to make a direct comparison of translocation induction by ^{239}Pu α particles and by ^{60}Co γ rays, with similar protracted exposures for both types of radiation.

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Reversible arrest of mouse 3T6 cells in G₂ phase of growth by manipulation of a membrane-mediated G₂ function

BESIDES replicating their genetic material and subsequently partitioning it between two daughters, cells of higher organisms also generally contain two distinct periods during their growth cycle in which they are engaged in neither activity. The first of these is a gap which occurs between the completion of one division and the onset of the next round of DNA synthesis, and is abbreviated as G₁. The ability to obtain stable populations of cells reversibly arrested in G₁ (refs 1–3) has made possible extensive investigation of this stage of the cell cycle⁴. The second gap (G₂) lies between the completion of DNA synthesis and the onset of mitosis. Chiefly because of the unavailability of techniques for reversibly holding cells at this stage, the possible functions of G₂ have remained largely unprobed⁵. I now report a method by which reversibly-arrested G₂ cells can be obtained and tentatively suggest one function of this phase as being the reorganisation of the cell surface in preparation for the start of mitosis.

It has become increasingly evident over the past few years that cell membranes are fluid regions^{6–9} within which movements of constituent macromolecules such as surface antigens¹⁰, membrane-bound enzymes⁸, and surface agglutinin receptor sites may occur^{11,13}. Such movements have been shown to be temperature dependent^{8,11,12}, occurring freely above 15 °C, but being greatly restricted when temperatures are lowered below this point^{2,11,13,14}. This has also been found to be a critical temperature range for the physical state of the lipid bilayer itself¹⁵, with a phase change occurring from a fluid to a crystalline gel as the temperature drops to about 15 °C. It is possible to influence the critical temperature at which this phase transition will occur. When this is done the temperature at which mobility of constituent macromolecules becomes restricted is comparably shifted¹¹. Such alterations have been accomplished by influencing membrane fatty acid composition^{11,19}; a higher degree of unsaturated fatty acids makes the achievement of a crystalline lipid state more difficult, so that membrane fluidity may be retained even at very low temperatures^{16–18}. This has been done by growing cells in a serum supplement from which lipid has been removed, thereby shifting the temperature dependence of the mobility of receptor sites for concanavalin A on 3T3 and SV40-transformed 3T3 cells¹¹. A similar technique was used in the present study.

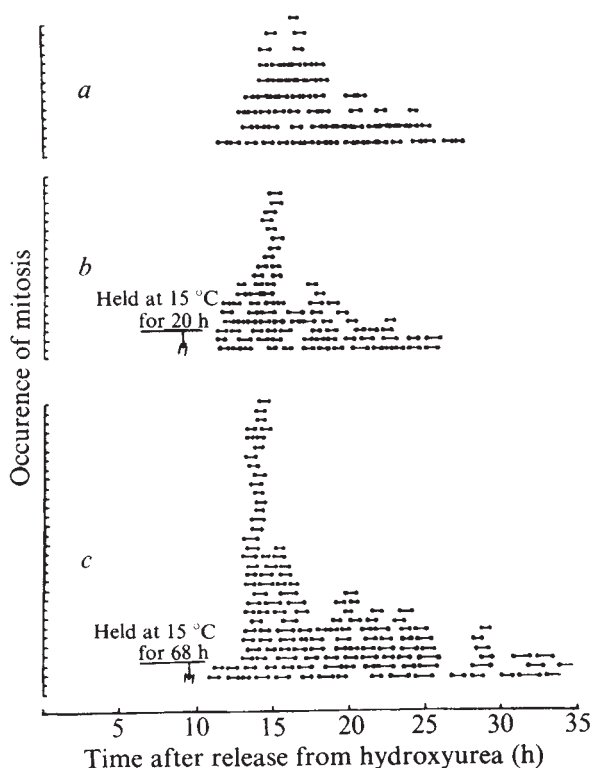


Fig. 1 Onset of mitosis after transferring cells from 15 to 37 °C. 3T6 cells were seeded at subconfluent densities (as cell-cell interactions might influence membrane transitions) in the absence of serum and incubated at 37 °C for 3 d. The G_1 arrested cultures thereby obtained were then transferred to rich serum conditions (10% foetal calf serum plus 5% calf serum), but in the presence of 2.88 mM hydroxyurea to hold the cells just at the border of entry into DNA synthesis. After incubation for 15 h in the presence of hydroxyurea, the cultures were washed and changed to fresh, serum-enriched, hydroxyurea-free medium. *a*, Filming was begun immediately after release from hydroxyurea. Incubation was at 37 °C throughout. *b*, Cultures were incubated at 37 °C for a further 9.5 h after hydroxyurea release and then transferred to 15 °C. After 20 h at 15 °C the cultures were shifted back to 37 °C and filming begun immediately. *c*, As in *b* except cultures were left at 15 °C for 68 h. In the filming analysis, individual cells were followed and the times at which they rounded up (early metaphase) and divided into two attached daughter cells (late telophase) both recorded. Each horizontal bar starts at the rounded and extends through the double-cell stage for one cell. Each bar, therefore, represents the first mitotic event for one particular cell. The bars are placed on the graph in accordance with the time of occurrence of these events, and if the mitoses of two cells overlap in time, then one is placed above the other.

It has also been suggested that lectin receptors on transformed cells may generally be more amenable to mobilisation than are those of their untransformed counterparts^{12,20} (see ref. 6 for review); although untransformed cells presumably take on a similar ease of surface rearrangement at about the time that they enter mitosis²¹. I have reasoned that if membrane reorganisation is a normal part of the progression of cells to mitosis, and if such reorganisation is contingent on an adequately fluid membrane environment, then interference with membrane fluidity should also prevent cells from entering mitosis. Since this sort of reorganisation may be expected to occur sometime between the completion of DNA synthesis and the onset of mitosis, then reversibly 'freezing' the membrane to prevent such movements from occurring, may be equivalent to reversibly arresting cells in the G_2 phase of growth.

I have attempted to 'freeze' movements within cell membranes by lowering the cultivation temperature of the mouse 3T6 cell line to 15 °C. Since a temperature decrease of this sort may be expected to have a variety of effects throughout the cell cycle^{16,22-24}, cells were first presynchronised to be as near as

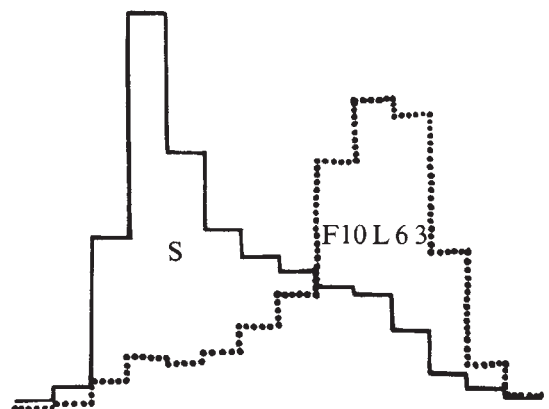
possible to the completion of DNA synthesis before the temperature was lowered. Experimental conditions were thus intentionally manipulated with the specific purpose of looking at membrane-related events in G_2 , rather than pursuing a general temperature effect on randomly growing cultures. Details of the presynchronisation are given in Fig. 1.

The mitotic responses of cells which had been held at 15 °C for various lengths of time and then shifted back to 37 °C are shown in Fig. 1. Here, mitoses were followed cinemicrographically, with the times of rounding up (early metaphase) and division into two attached cells (late telophase) recorded and plotted for each individual mitotic event. Figure 1*a* shows the profile for onset of mitosis after release of cells from hydroxyurea block and cultivation without interruption at 37 °C. The first mitoses occurred at about 12 h after release, with a broad peak between 14 and 19 h. If, at 9.5 h after release from hydroxyurea, cells were transferred to 15 °C for either 20 h (Fig. 1*b*) or 68 h (Fig. 1*c*) and then returned once again to 37 °C, the ensuing mitotic pattern was altered as shown. In both cases there was a lag of about 2 h before any mitoses were apparent at all. This time lag was not shortened by longer periods at the low temperature, and was followed by a very sharp mitotic burst occurring about 3-4 h after return to 37 °C. This burst was intensified by the longer period at the low temperature (Fig. 1*c*). It would seem from these observations that cells are being arrested, and not just retarded, in their movement through the G_2 phase, and that this arrest is reversible.

I attempted to confirm the conclusion that cells were being held in G_2 by low temperature by measuring the DNA content per cell on a BDH flow microfluorimeter. This is shown in Fig. 2 where the amount of DNA per cell in randomly growing cultures (S) is compared with that of cultures which had been presynchronised and then transferred to 15 °C (F10L63). There is a marked shift of the distribution in the F10L63 cells to the G_2 region. It can be roughly calculated from these distributions that in randomly growing cultures about 17% of the cells are in G_2 , whereas in the F10L63 the G_2 population accounts for over 80% of the cells.

If the G_2 arrest is a function of the fluidity of some cellular membrane system, then the maintenance of high fluidity, even at low temperature, should enable the cells to proceed to mitosis even at 15 °C. As has been mentioned above, this can be attained by altering fatty acid compositions by cultivating the cells with a delipidated serum supplement. We treated foetal calf serum as detailed in Fig. 3 and then grew 3T6 cells in this

Fig. 2 DNA contents per cell of randomly-growing and cold-arrested 3T6 cells. Either randomly-growing cells (S) or cells which had been held at 15 °C for 63 h (F10L63) (as described for Fig. 1), were analysed for their DNA contents. Cells were fixed and stained with acriflavine according to the method of Tobet *et al.*³² and the fluorescence per cell determined on a BDH flow microfluorimeter. The abscissa is in arbitrary fluorescence units, proportional to the amount of DNA, per cell and the ordinate represents the number of cells in that fluorescence channel. Between 1×10^6 and 2×10^6 cells were counted for both S and F10L63 cultivation conditions.



delipidated supplement, with an additional supply of biotin, (an essential cofactor for the biosynthesis of fatty acids) through several transfers at 37 °C. The fatty acids of cells which had been grown in normal serum and those which had been cultivated in the delipidated serum ('Delip' cells) were extracted²⁶ and analysed on a polyethylene glycol-adipate gas-liquid chromatography (GLC) column.

It was found that the proportion of saturated palmitate and stearate fatty acids in the 'delip' cells had decreased about threefold, whereas the unsaturated oleate plus elaidic fatty acids had proportionately increased about 2.5-fold compared with cells which had been grown in whole serum. To test whether this treatment also resulted in the release of cells from the low temperature G₂ block, the 'delip' cells were presynchronised and then shifted to 15 °C (Fig. 1). The DNA content/cell profiles for randomly growing 'delip' cells at 37 °C and cells held at 15 °C are shown in Fig. 3. Cells with the altered fatty acid composition were not held in G₂ at 15 °C. This was in agreement with the finding that the cell number of such cultures gradually increased at 15 °C, although no similar increase in number could be detected for cells which had been grown in whole serum.

I conclude that there is a temperature-dependent transition point in the G₂ phase of normal cell growth which is related to the fatty acid composition of some cell membrane system. I suggest that the G₂ function involved may be an obligatory surface rearrangement before mitosis, and that this rearrangement can be reversibly inhibited by decreasing membrane fluidity. Although fatty acid replacement experiments strongly implicate a fluid membrane function, one cannot identify definitively, from the present experiments, which of the cellular membrane systems may be involved^{14,16}. Careful tailoring of membrane fatty acid compositions so as to adjust critical temperature transitions points to more physiological ranges should help both in elucidating this question as well as in providing a possible new method for sharply synchronising cells in the G₂ phase of growth.

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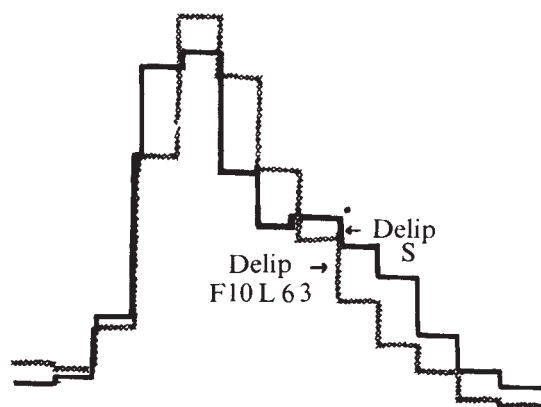


Fig. 3 DNA contents per cell of 3T6 cells with altered fatty acid composition. As for Fig. 2 except using cells which had been grown with a delipidated serum supplement. 100 ml of foetal calf serum were stirred with 100 ml acetone at 4 °C for 3 h. After centrifugation at 1,800g for 30 min, the cloudy yellow supernatant was discarded and the pellet resuspended in 150 ml chilled acetone and stirred in the cold overnight. This was then centrifuged, the supernatant discarded, and the sediment collected and dried under vacuum; this yielded 3.52 g of a white powder which could be stored for several months at -20 °C. For use in cell cultivation, 600 mg of the powder were suspended in 10 ml of 0.01 M acetate at pH 4.0 and then dialysed extensively against the acetate buffer in the cold. The suspension was then spun down at 1,800g for 2 h and the supernatant collected. (This was the delipidated serum and it contained ~ 16.5 mg protein per ml.) The delipidated serum was filter-sterilised before use. S, Randomly growing cultures at 37 °C. F10L63, cells held at 15 °C for 63 hours.

Independent routes for Na transport across dog red cell membranes

PARKER and SNOW¹ have shown that extracellular ATP produces an apparently nonspecific and reversible increase in the cation (Na⁺ and K⁺) permeability of dog red cells, as a result of which the cells swell gradually during suspension in an isosmolar physiological bathing medium containing ATP. This effect contrasts markedly with the usual dependence on cell volume of cation fluxes in this type of red cell²⁻⁶ which, unlike most mammalian erythrocytes, is close to ionic equilibrium with plasma⁶⁻⁷, and lacks an ouabain-sensitive ion pump^{8,9}. Exogenous ATP was found to influence the surface properties of chick embryo fibroblasts¹⁰, and also cause volume changes and cation imbalances in ascites tumour cells¹¹, and it has been suggested¹¹ that ATP alters the passive permeability of the membrane to Na⁺ and K⁺ rather than the cation pumping mechanism *per se*. Effects of ATP on the membrane transport characteristics of various tissue culture cell lines have been studied^{12,13}, and it has been proposed¹² that the translocation of ATP itself may be linked with ion movement across membranes.

I have now measured ²⁴Na⁺ uptake in dog red cells suspended in various media with and without extracellular ATP, and have found that the steady-state Na⁺ influx, referred to cell volume in control isotonic conditions, is not changed significantly by swelling the cells in hypotonic media. In the presence of ATP, however, Na⁺ permeability is greatly increased whether the cells are allowed to swell naturally or are shrunk by the osmotic effects of sucrose; in these circumstances it was necessary to estimate unidirectional Na⁺ fluxes from the half times for ²⁴Na⁺ equilibration⁶. The sum of the Na⁺ fluxes measured separately in cells suspended in isosmolar media

containing ATP, and in cells suspended in hyperosmolar media (+ 100 mM sucrose) in the absence of ATP, is equal to the Na^+ flux in ATP-treated cells after they have been shrunk. Shrinkage of the cells was carried out in the presence of ATP in media containing sucrose (100 mM). This indicates that the ' Na^+ channel' opened in the presence of ATP, when the cells are swollen, is not closed by shrinking the cells, and that the increase in the permeability to Na^+ normally brought about by shrinking the cells occurs by way of a route which is independent of and parallel to the pathway opened in the presence of extracellular ATP.

Quantitative measurements of $^{24}\text{Na}^+$ uptake, relative cell volume and intracellular Na^+ and K^+ concentrations were made from a series of single samples of packed cells spun down during the uptake of $^{24}\text{Na}^+$. ^{131}I -human serum albumin was used as the extracellular marker. Known volumes of packed cells were sampled with a Hamilton gas-tight syringe fitted with a Chaney adaptor. Details of the method have been described previously^{5,6,14}.

Figures 1 and 2 illustrate respectively the differences in the haemoglobin concentration (Hb) and the rate of uptake of $^{24}\text{Na}^+$ by cells in various experimental conditions. Cells suspended in media containing 25 mM and 50 mM less NaCl than the control isosmolar medium swelled by $13.1 \pm 0.7\%$ and $30.9 \pm 0.8\%$, respectively, and were at constant cell volume during the uptake of $^{24}\text{Na}^+$. In these conditions Na^+ influx was not altered significantly. By contrast, although the average volume of cells exposed to hyposmolar conditions (-50 mM NaCl) was similar to that of cells exposed to 2 mM ATP in an isosmolar medium (Fig. 1), in the latter group there was a 68-fold increase in Na^+ flux and a net uptake of Na^+ (Table 1).

When cells were suspended in a solution containing 100 mM sucrose, in addition to the usual constituents, they shrank by an average of $24.0 \pm 0.8\%$ and the Na^+ flux increased by a factor of 44 (Table 1). Although treatment with ATP had no significant effect on cell volume in the presence of 100 mM sucrose, the rate of Na^+ exchange was, however, increased even further to 395 ± 46 mEq per l cells (isosmolar cell volume) per h after ATP-treated cells had been shrunk. It is unlikely that the agreement between this value and the sum (437 ± 34 mEq per l cells per h) of the fluxes measured independently was fortuitous, as virtually the same findings were produced by separate experiments with red cells from two other dogs.

A secondary outcome of the present work was the lack of any discrepancy between the equilibrium levels of intracellular and extracellular specific activity of $^{24}\text{Na}^+$ in swollen cells treated with ATP. This indicated that the apparent sequestration of intracellular Na^+ in shrunken cells which was found in previous studies^{5,6} was in fact the result of partial dehydration of the cells.

The effects of exogenous ATP and cell shrinkage on Na^+

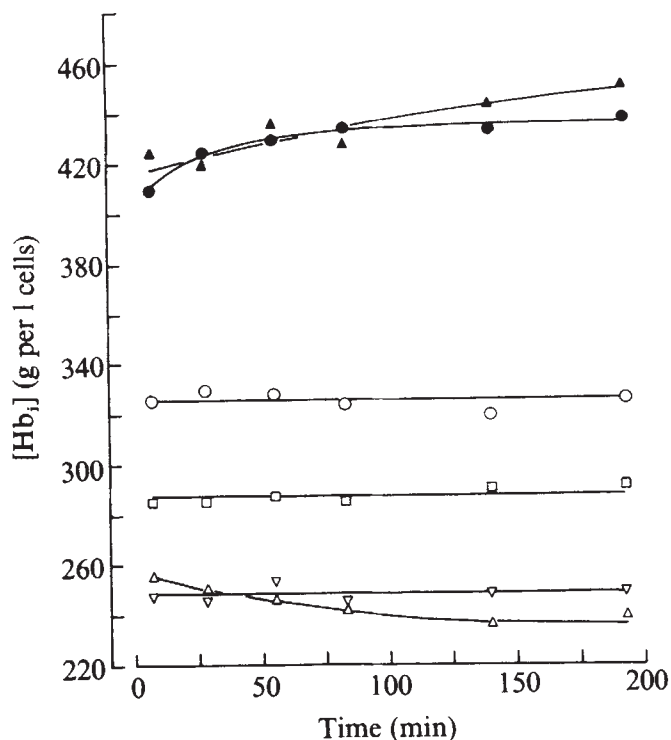


Fig. 1 Haemoglobin content of dog red cells during the uptake of $^{24}\text{Na}^+$ illustrated in Fig. 2. These data provide a measure of cell volume in the different media relative to that in control isosmolar conditions: NaCl, 137.2 mM; KOH, 5.0 mM; TES (N-tris [hydroxymethyl] methyl-2-aminoethane sulphonic acid), 5.0 mM; Na_2HPO_4 , 5.2 mM; NaH_2PO_4 , 0.8 mM; glucose, 5.0 mM; and 1% (w/v) bovine serum albumin (Armour, Cohn fraction V). Ca^{2+} and Mg^{2+} were omitted as they greatly inhibited the effect of ATP (ref. 1 and B. C. E., unpublished). pH of solutions containing ATP was adjusted to 7.2–7.4 with KOH (1.2 mM per mM ATP). Washed red cells were preincubated (where appropriate) in an isosmolar medium with 2.0 mM ATP for 2 h at 38°C , then washed and resuspended in the same solution ($\pm$ sucrose 100 mM) at haematocrit values of about 10%. Control isosmolar, hyperosmolar and hyposmolar groups of cells were treated in a similar fashion with the appropriate bathing medium. Finally, all groups were gently shaken at 38°C for 75 min in the presence of ^{131}I -albumin before the start of $^{24}\text{Na}^+$ uptake. $\blacktriangle$, +2 mM ATP, +100 mM sucrose; $\bullet$, +100 mM, sucrose; $\circ$, control; $\square$, -25 mM NaCl; $\square$, -50 mM NaCl; $\triangle$, +2 mM ATP.

fluxes are additive, implying that separate pathways are involved. There is, however, a common factor between the processes controlling the two states of increased permeability to Na^+ , as it has been proposed⁸ that phosphoglycerate kinase controls the dependence on cell volume of Na permeability in

Table 1 Na^+ fluxes in dog red cells as a function of cell volume in the presence and absence of ATP (2 mM)

Experimental conditions	Hb _i (g per l cells*)	Na^+_{i} (mEq per l cells*)	K^+_{i} (mEq per l cells*)	Normalised† Na^+ flux (mEq per l cells per h)
Control	$325.2 \pm 1.4^\dagger$	$97.6 \pm 0.3^\dagger$	$4.3 \pm 0.1^\dagger$	$3.9 \pm 0.1^\S$
NaCl, -25 mM	287.6 ± 1.1	86.6 ± 0.4	3.9 ± 0.1	$3.7 \pm 0.1^\S$
NaCl, -50 mM	248.5 ± 1.1	75.0 ± 0.1	3.4 ± 0.1	$3.9 \pm 0.1^\S$
ATP, +2 mM	243.5 ± 4.0	107.0 ± 1.0	5.1 ± 0.1	$265 \pm 20^\P$
Sucrose, +100 mM	427.9 ± 4.1	108.2 ± 0.8	5.4 ± 0.2	$172 \pm 28^\P$
Sucrose, +100 mM ATP, +2 mM	433.8 ± 5.0	99.6 ± 1.7	4.6 ± 0.1	$395 \pm 46^\P$

*Measured cell volume.

†Mean $\pm$ s.e. of six determinations in each column.

‡Fluxes referred to 1 l cells in control isosmolar conditions.

§ Na^+ influx given by the ratio of the slope of the regression line fitted to the uptake of $^{24}\text{Na}^+$ (Fig. 2) and the extracellular specific activity of $^{24}\text{Na}^+$.

¶ Na^+ flux calculated from the rate constant of the exponential uptake of $^{24}\text{Na}^+$ (Fig. 2) using a two-compartment model⁸. s.e. of the fluxes were estimated from the s.e. of the component parameters.

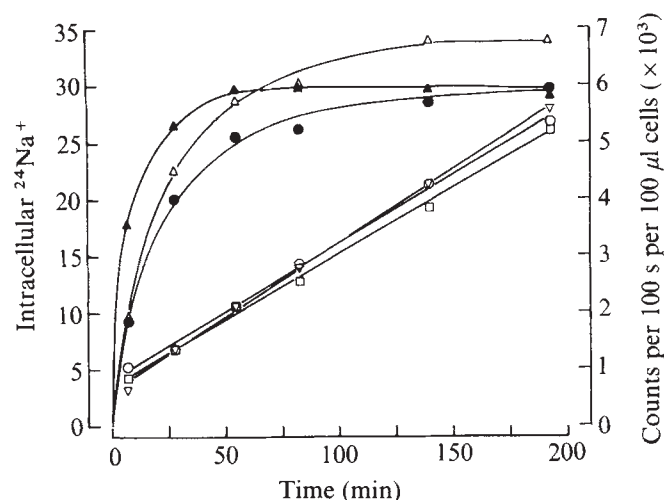


Fig. 2 Uptake of $^{24}\text{Na}^+$ by dog red cells in the conditions described in Fig. 1. Symbols as in Fig. 1. Right hand ordinate scale expanded fivefold for influx of $^{24}\text{Na}^+$ in control isosmolar conditions ($\circ$) and in hyposmolar media (-25 mM NaCl , $\square$ and -50 mM NaCl , ∇). In groups $\bullet$, $\blacktriangle$, $\triangle$ (all left-hand ordinate) and $\circ$, initial values of extracellular specific activity were almost the same (about 3.65×10^6 counts per 100 s per mEq Na^+); in groups $\square$ and ∇ the values were 4.23 and 5.32×10^6 , respectively. Half times for the exchange of $^{24}\text{Na}^+$ were 7.6 ± 0.8 min ($\blacktriangle$), 18.6 ± 3.0 min ($\bullet$) and 20.4 ± 1.2 min ($\triangle$), compared with about 780 min for groups ∇ , $\circ$ and $\square$. Na^+ fluxes estimated from these plots are listed in Table 1.

the dog red cell; and this enzyme catalyses one of the ATP-producing reactions in the glycolytic sequence.

There is evidence¹ that exogenous ATP can alter the metabolic rate of dog red cells; however, it would seem that hydrolysis of the nucleotide is not providing a source of energy for the maintenance of the Na^+ channel, as ouabain (up to 10^{-3} M) has no inhibitory effect on the enhanced Na^+ flux in ATP-treated cells¹. Note also that the ATP effect is probably not mediated by the removal of membrane-bound divalent cations, as up to $5 \times 10^{-3}\text{ M}$ EDTA, which chelates Ca^{2+} and Mg^{2+} more strongly than does ATP^{15,16}, has no effect *per se* on cell volume or Na^+ transport in dog red cells; neither does it interfere with the action of ATP in cells suspended in Ca^{2+} - and Mg^{2+} -free media (B. C. E., unpublished). Furthermore, it seems that ADP and other nucleotides are virtually without effect on cation permeability in the dog red cell¹.

The actual mechanisms of the volume-dependent and ATP-induced permeability changes are still unclear^{1,4,5,11,12,17,18}; however, because of the large and to some extent cooperative increase in Na^+ flux caused by either ATP¹ or cell shrinkage⁶ it is tempting to refer to Na^+ channels being switched on in the two circumstances. It would be extremely interesting if the properties of these channels were to bear any resemblance to the cation channels that have been characterised in nerve preparations¹⁹. Preliminary experiments, however, have indicated that tetrodotoxin (10^{-6} g ml^{-1} , pH 7.0) has no effect on the Na^+ channel opened in shrunken cells.

Obviously, cells are not going to encounter extracellular ATP concentrations of the order of $1\text{--}2\text{ mM}$ *in vivo*. It is clear, however, that ATP provides a specific way of perturbing membranes that gives rise to marked changes in permeability characteristics, not only of dog red cells but of a variety of cell types.

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State of molecular motion of cholesterol in lecithin bilayers

CHOLESTEROL is a major constituent of many biological membranes. Although its exact function is not completely understood, a large number of studies have indicated that cholesterol can affect the packing of lipid hydrocarbon chains, both in model and in biological membranes¹. The interaction is envisaged to be such that above the thermal phase transition temperature (T_c), cholesterol inhibits flexing of the hydrocarbon chains, whereas below T_c it prevents the chains from crystallising into the rigid α gel²⁻⁶.

Rothman and Engelman have argued⁷ that the interactions between cholesterol and the phospholipid hydrocarbon chains in a lipid bilayer are primarily steric in nature, and the dramatic effects of cholesterol on the motional state of a phospholipid bilayer resides in the relative cross sectional area of the ring system (the nucleus) compared with that of the aliphatic tail. As the cross-sectional area of the nucleus is approximately twice that of the tail, steric interactions involving these parts of the molecule are thought to be different. If one supposes that in cholesterol-containing bilayer membranes, the upper part of the fatty acid chain is contiguous with the rather bulky cholesterol nucleus and the lower part of the chain with the narrower cholesterol tail, it seems plausible that cholesterol can serve to limit the number of accessible chain conformations along the upper part of the fatty acid chain while simultaneously increasing the number of accessible conformations to the lower part of the chain.

The question of cholesterol mobility is intriguing and important. Although the model of Rothman and Engelman⁷, which views the cholesterol nucleus as being rather immobile and the tail as highly mobile, seems reasonable, it is primarily based on model building. Several nuclear magnetic resonance studies^{6,8,9} disagree with this notion and claim that the cholesterol molecule as a whole, including the tail, is highly immobile, as a consequence of some specific 1:1 phospholipid-cholesterol complex formation. Unfortunately proton magnetic resonance spectra of cholesterol containing bilayer vesicles are not amenable to unambiguous interpretation. The spectrum is dominated by the broad methylene and methyl resonances of the phospholipid chains (Fig. 1a). Although cholesterol accounts for 25% of the total number of protons in a 2:1 lecithin-cholesterol mixture, none of its resonances could be discerned.

The molecular mobility of cholesterol in lipid bilayers is best ascertained by observing its proton magnetic resonance spectrum in conditions in which it is free of interference from the more intense hydrocarbon chain signals of the phospholipid molecules. The methylene and methyl resonances of the phospholipid chains can be suppressed if the fatty acid chains become totally deuterated. We have accordingly synthesised a series of $\sim 99\%$ deuterated lecithins for this purpose¹⁰. Figure 1b shows the corresponding spectrum of cholesterol in the deuterated L- α -dipalmitoylphosphatidylcholine (DPL) bilayer vesicles containing one part of cholesterol to two parts of lecithin at 75°C . A cholesterol spectrum with rather well-defined features is obtained.

The most notable feature of the cholesterol spectrum is the

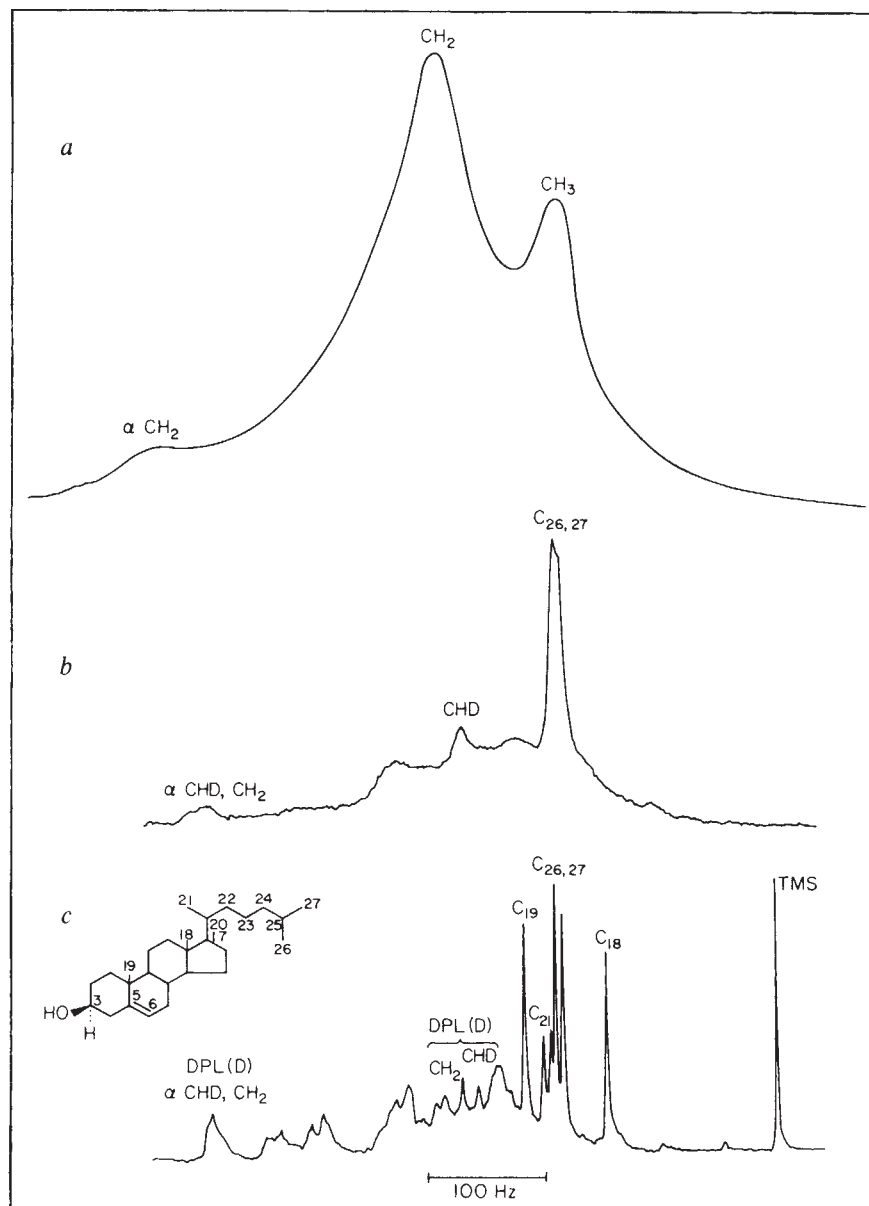


Fig. 1 Proton magnetic resonance (220 MHz) spectra of hydrocarbon chain protons of DPL vesicles containing cholesterol at 50 °C (a); cholesterol in deuterated DPL vesicles at 75 °C (b); sample *b* after freeze-drying and redissolved in deuteriochloroform containing TMS at 20 °C (c). DPL-cholesterol molar ratio $\sim 2:1$ for all samples.

rather sharp, intense doublet which stands out amidst the broad background at -0.9 p.p.m. We assign this resonance by comparison with a spectrum of the same sample dissolved in CDCl_3 (Fig. 1c)¹¹. There are five methyl groups in cholesterol and each of these shows up clearly in the chloroform spectrum (see spectral assignment indicated in Fig. 1c). Reference to this spectrum suggests that the sharp cholesterol resonance observed in the vesicle spectrum is to be assigned to the terminal C_{26} , C_{27} methyl groups. This assignment is confirmed by the field independent splitting, which is due to spin-spin coupling with the C_{25} methine proton. If this assignment is correct, essentially all the C_{26} , C_{27} methyl groups are contributing to this signal.

The remaining methyl resonances seem to be much broader, which indicates that their segmental motion is much more restricted than that of the isopropyl group of the tail. The degree of segmental mobility of cholesterol can be ascertained from the overall intensity of the cholesterol resonances appearing in the high resolution spectra, including the broader signals. Accurate intensity measurements of broad resonances in a high resolution spectrum are difficult, as the choice of baseline is always open to debate. Nevertheless, on a basis of the comparison of the total integrated intensity of the cholesterol resonances relative to that of the choline proton signal in our deuterated DPL, we conclude that no less than 70% of the cholesterol resonances including those of the C_{26} , C_{27} methyl

groups are observable at 75 °C. As the cholesterol sidechain protons correspond to only 50% of the total number of protons in the molecule, at least some of the cholesterol ring protons must be contributing to the spectrum. Thus cholesterol, when incorporated into a lipid bilayer, does not become totally immobilised. These results provide strong support for the Rothman-Engelman model.

The effect of temperature on the cholesterol spectrum is shown in Fig. 2. As the temperature is lowered the C_{26} , C_{27} methyl resonances show a gradual broadening, the linewidth increasing from ~ 13 Hz at 83.4 °C to ~ 23 Hz at 54.5 °C. These observations, we believe, reflect the expected gradual increase in the segmental order and/or a decrease in mobility of the tail as the temperature is decreased. It is significant, however, that even at 26 °C a cholesterol spectrum could still be discerned, although the resonances were now very broad. There is thus significant residual segmental motion of the cholesterol molecule even at temperatures 11 °C below the thermal phase transition temperature of DPL¹⁰. In contrast, small DPL vesicles revealed no high resolution hydrocarbon chain resonances at this temperature in the absence of cholesterol.

Engelman and Rothman⁴ have also proposed that a phase boundary occurs in DPL-cholesterol mixtures at 20 °C in the region near 33 mol % cholesterol. To ascertain the existence of this or a similar phase boundary at higher temperatures, we have examined mixed cholesterol-lecithin systems at two

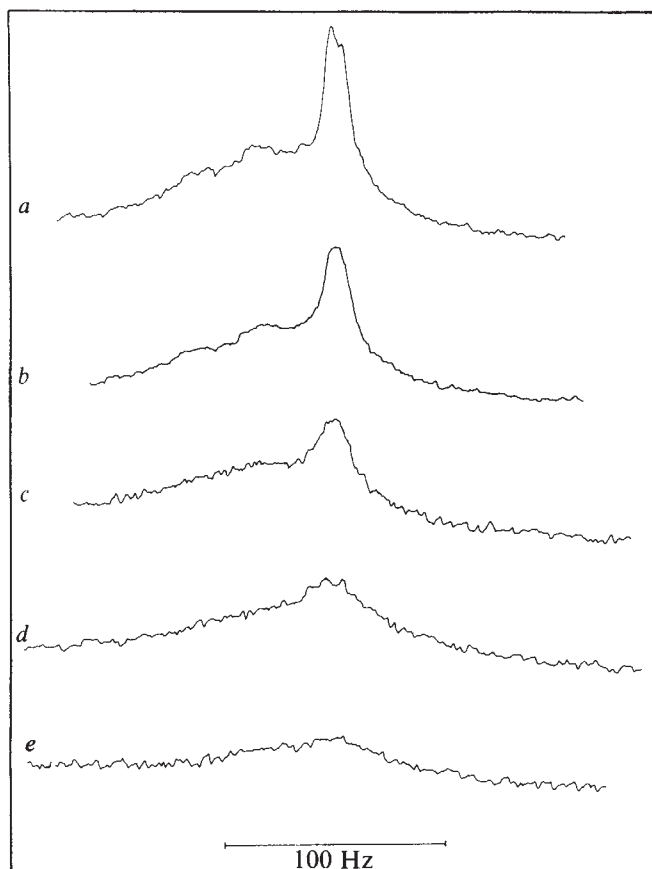


Fig. 2 Effect of temperature on 100 MHz proton magnetic resonance spectra of cholesterol in deuterated DPL vesicles. DPL-cholesterol molar ratio ~2:1. a, 83.4; b, 69.5; c, 54.5; d, 36.5; e, 26 °C.

concentrations corresponding to DPL-cholesterol molar ratios of 2:1 and 1:1. Proton magnetic resonance measurements of these two bilayer systems at 83.4 °C (and at other temperatures) revealed no significant difference between the spectra for the two samples, except for an intensity deficiency in the cholesterol C_{26} , C_{27} methyl peaks for the sample more concentrated stoichiometrically in cholesterol. Whereas intensity measurements of the sharp cholesterol methyl signals relative to that of the choline methyl peak of the lecithin indicated that all the cholesterol methyl signal intensity expected is being observed for the 2:1 mixture, 30% of the cholesterol methyl intensity is not accounted for in the 1:1 mixture. This result suggests the existence of a phase boundary in lecithin-cholesterol bilayers at a cholesterol concentration of ~40%.

In summary, we have obtained experimental evidence for a differential mobility of the cholesterol nucleus and its aliphatic tail when cholesterol is incorporated into lecithin bilayer vesicles. The motion of the isopropyl moiety of the tail was shown to be essentially unrestricted, and exhibited a high degree of mobility even at temperatures well below the thermal phase transition of the phospholipids into which it is incorporated. We have also obtained evidence for the existence of a phase boundary in mixed cholesterol-lecithin systems in the region near 40 mol % cholesterol.

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Effects of structural defects in sonicated phospholipid vesicles on fusion and ion permeability

ULTRASONICATED lecithin-water dispersions, consisting of single-walled bilayer vesicles, are used as a model for the phospholipid bilayer in biomembranes and have the unique advantage of a well-defined inside and outside^{1,2}. The system is well suited, therefore, for studies of transport of solutes across the bilayer membrane³. Fusion of these structures may also be used as a model for cell fusion, endocytosis and related phenomena^{4,5}.

Small sonicated vesicles, because of their large surface curvature, are known to exhibit different physical properties from large bilayer vesicles and multilamellar liposomes². In particular, there is a tendency for the size distribution of a vesicle suspension to change spontaneously by the process of vesicle fusion in certain temperature conditions⁶. As the definition and control of this model membrane system are important, factors influencing the stability of bilayer vesicles are of interest. Here we describe an annealing phenomenon which we have found to be necessary for the stability of vesicle suspensions.

Variations in the vesicle size distribution can be monitored by changes in the proton magnetic resonance spectrum. Previous studies have demonstrated that for large vesicles the proton magnetic resonance spectrum of the hydrocarbon chain protons is broadened compared with that for small sonicated vesicles, together with a concomitant loss of intensity². In addition, small sonicated vesicles exhibit distinct inner and outer choline methyl signals at high magnetic fields because of different surface curvatures between the two halves of the bilayer membrane⁷. This chemical shift non-equivalence enables perturbations on the inner and outer halves of the bilayer to be monitored separately. Large single-walled vesicles which do not possess this structural difference between the two halves of the membrane, however, do not exhibit this chemical shift non-equivalence, but the addition of a paramagnetic shift reagent to the external solution after preparation results in well separated choline signals provided the bilayer membrane is impermeable to the paramagnetic ion⁸.

Small sonicated phospholipid bilayer vesicles are usually obtained by prolonged sonication of a phospholipid dispersion at high sound intensity. In view of the high power input to the sample, the local temperature of the solution frequently reaches well above the thermal phase transition (T_c) of the lipids. Recently we have found that vesicles with a controlled range of average sizes can be prepared directly by mild sonication at low temperatures ($< T_c$), but that subsequent heating of the resultant sonicated lecithin dispersion to a temperature above T_c is an absolute prerequisite for obtaining a bilayer structure which is impermeable to ions. Moreover, there was a pronounced tendency for the sonicated vesicles to fuse, with the rate of fusion depending strongly on whether they have been annealed or not. These observations, we feel, have bearing on a number of reports regarding the rapid fusion of small sonicated bilayer vesicles^{6,9}.

Figure 1 illustrates both the effects of annealing on the permeability of bilayer vesicles to Eu^{3+} ions and on the rate of vesicle fusion. When $\text{Eu}(\text{NO}_3)_3$ was added in isotonic conditions

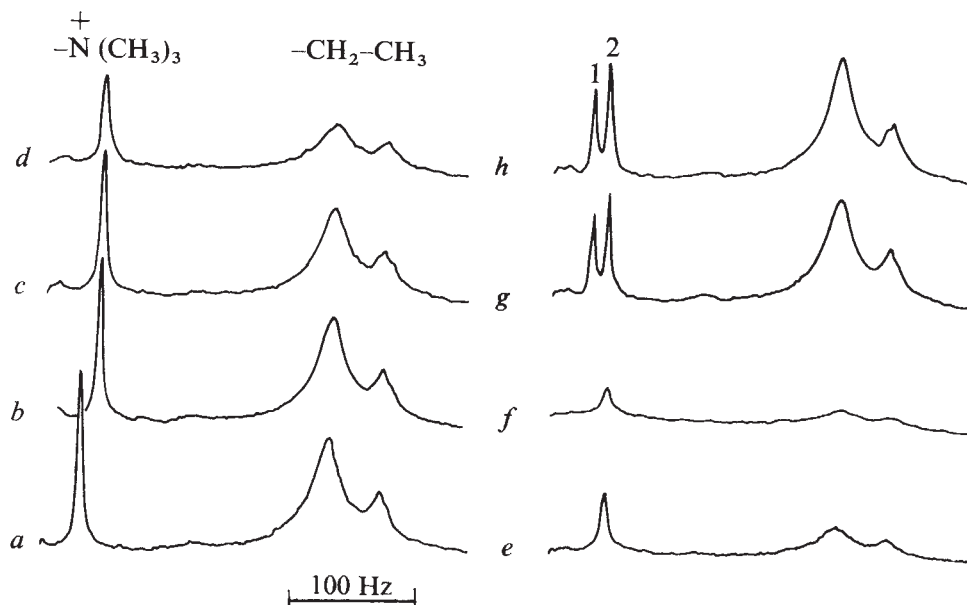


Fig. 1 DSL (2.5 w/v%), purified by a silicic acid column chromatography, was sonicated at high power in a 5 mM solution of $\text{La}(\text{NO}_3)_3$ in D_2O using a Branson sonicator with Ti microtip. To maintain the solution below T_c during ($\sim 56^\circ\text{C}$) sonication, it was cooled externally by an ice-water bath, and an alternating sonication sequence of 30-s periods of sonication and 30-s intervals was used. After a total of 15 min a practically transparent solution was obtained. This was divided into eight 5-mm nuclear magnetic resonance sample tubes, which were kept at different annealing temperatures (see below) for 30 min. The samples were then brought to room temperature (23°C) and equal volumes of 5 mM $\text{Eu}(\text{NO}_3)_3$ solutions were added immediately to all the samples, except for the control sample (a) to which a 5 mM $\text{La}(\text{NO}_3)_3$ solution was added. After thorough mixing by shaking, all samples were kept at 69°C , the nuclear

magnetic resonance probe temperature, until the nuclear magnetic resonance spectrum was recorded. The latter step was introduced to fix the state of all the samples before recording spectra. Fourier transform ^1H nuclear magnetic resonance spectra were obtained with a Varian XL-100 spectrometer. The temperatures ($^\circ\text{C}$) at which the samples were kept before addition of the La salt solution (the annealing temperature) were: a, 23; b, 23; c, 42; d, 49.5; e, 52.4; f, 55.6; g, 59.2; h, 63. The spectral assignments indicated correspond to the choline methyl protons ($-\text{N}^+(\text{CH}_3)_3$) and the methylene and methyl proton of the hydrocarbon chain (CH_2 , CH_3). 1 and 2, Choline methyl signals associated with the inside and outside halves of the bilayer, respectively. Measurements of the signal intensities of the choline methyl resonances for samples a, b, and h indicate that in excess of 90% of the expected intensities are being observed.

to samples of L- α -distearoylphosphatidylcholine (DSL) or L- α -dipalmitoylphosphatidylcholine (DPL) at room temperature after sonication, the proton magnetic resonance spectrum of these vesicles above T_c exhibited only one methyl signal for both the inner and outer choline groups. The spectral position of this choline signal was shifted to higher fields relative to that in a similar preparation containing only $\text{La}(\text{NO}_3)_3$ in both the intra and extravascular solutions, indicating leakage of Eu^{3+} ions into the internal compartment of these vesicles in these conditions. In contrast, when another portion of the same sonicated preparation was heated above T_c before Eu^{3+} ions were added to the solution at room temperature, the proton magnetic resonance spectrum of these vesicles revealed two choline methyl peaks. A more detailed study of this phenomenon involving preheating samples at various temperatures (Fig. 1) after sonication and cooling of the sample back to room temperature before the addition of the shift reagent has led us to conclude that annealing of the vesicles above the thermal phase transition is absolutely essential to obtain bilayers which are impermeable to ions.

Bilayer vesicles which have not previously been annealed above T_c have been found to undergo extensive fusion. The fusion rate increases with increasing temperature and seems to reach a maximum at the thermal phase transition temperature. This vesicle fusion is manifested in the proton magnetic resonance spectra in a pronounced way. There is a conspicuous absence of intensity in the high resolution spectra. As the observed spectra do not seem to be significantly broadened from those for small sonicated bilayer vesicles, this intensity anomaly may be caused by polydispersity of the final vesicle suspension as a result of different extents of annealing in the original preparation.

Our experiments also revealed a correlation between the permeability of these bilayers to ions and their tendency towards fusion. As annealing starts around T_c , the bilayer membrane becomes less permeable to Eu^{3+} ions. The fusion rate also

goes down abruptly. Because of the relationship between annealing and fusion, kinetic experiments on annealing are somewhat difficult to interpret. Nevertheless it seems that annealing is complete within 2 h if it is carried out at 3°C above T_c . At higher temperatures the annealing is even faster; for example, annealing is completed within 10 min at 10°C above T_c . After the vesicles are annealed, fusion was extremely slow. For annealed samples of DPL vesicles, proton magnetic resonance and turbidity measurements revealed no evidence for fusion, even when they were kept for 16 h at 42°C (T_c). Thus the fusion of phospholipid bilayer vesicles depends strongly on the history of the sample with respect to annealing.

The above observations can be accounted for by structural defects within the bilayer structure after sonication below T_c . The high permeability of unannealed structures towards ions may be caused by imperfect boundaries between otherwise homogeneous lipid domains. In fact, we have evidence for structural differences between annealed and unannealed bilayer samples below T_c . Figure 2 shows proton magnetic resonance spectra of a DSL sample before and after annealing (15 min at 67°C) and recorded at 50°C (below T_c). Between the unannealed and the annealed sample we observed $\sim 50\%$ increase in the intensities together with a pronounced spectral sharpening of the hydrocarbon chain signal. Identical observations were obtained for DPL. These observations suggest that the unannealed bilayer vesicle below T_c consists of a discontinuous arrangement of highly ordered lipid domains. Unannealed bilayer vesicles could also be permeable to Eu^{3+} because of a highly fluid, but homogeneous bilayer structure. This possibility seems unlikely since such a bilayer membrane would be expected to yield sharper resonances before, rather than after, annealing—just the reverse of what is observed (Fig. 2). Electron microscopic studies also support the idea of cracks in the bilayer structure between well ordered lipid domains. It has not been possible so far to obtain electron micrographs for unannealed phospholipid vesicles by the negative staining

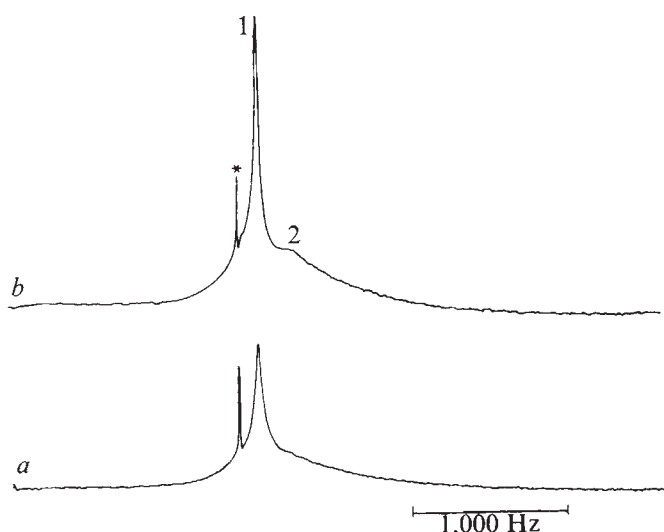


Fig. 2 Fourier transform ^1H nuclear magnetic resonance spectra (100 MHz) of the sonicated DSL solutions (2.5 w/v%) in D_2O containing 5 mM $\text{La}(\text{NO}_3)_3$. Procedure of sonication as described in Fig. 1. Sample *b* was the same as *a* except that it has been annealed for 15 min at 67°C . Nuclear magnetic resonance measurements were made at 50°C , which is below the phase transition temperature of DSL ($\sim 56^\circ\text{C}$), to prevent further annealing during the nuclear magnetic resonance measurement. 1 and 2, Choline methyl and aliphatic methyl and/or methylene signals respectively. *Residual H_2O signal, which was partially saturated by the fast pulsing cycle.

method, presumably because the staining solution leaks through these regions in the bilayer structure.

The above observations regarding the relationship between annealing and fusion were observed for DSL, DPL and $\text{L}-\alpha$ -dimyristoylphosphatidylcholine (DML). Similar results were obtained irrespective of whether the studies were carried out in pure D_2O or in NaCl solutions, although the vesicle dispersions in these cases were not as clear as in the presence of La^{3+} ions, suggesting that electrostatic surface conditions may influence the formation of vesicles during the sonication procedure. There is a pronounced difference between purified (by silicic acid column chromatography) and unpurified samples in that the fusion rate is much higher for the purified lipids. Rapid fusion of DML and DPL vesicles at the phase transition temperature of these lipids has been reported^{6,9}. The problem of annealing, however, especially its effect on fusion, was unknown.

Measurements on mixed lipid systems show analogous results. For a mixture of DPL and DSL (1:1 by weight) the appropriate annealing temperature and the temperature associated with the maximum fusion rate was found to be about 49°C , midway between T_c for the respective pure lipids, suggesting ideal mixing within the lipid domains. Other mixed lipid systems, such as DPL with $\text{L}-\alpha$ -dipalmitoylphosphatidylethanolamine (DPE), cholesterol or sphingomyelin (bovine brain), show similar behaviour.

In support of our proposal of rapid fusion of unannealed bilayer vesicles, comparative absorbance measurements on mixtures of DSL and DPL vesicles indicated that such mixtures of lipid vesicles exhibit distinct differences in their behaviour on heating, depending on whether the vesicles were annealed or not before mixing of the otherwise homogeneous suspensions. Only when both the DSL and DPL vesicle suspensions were mixed in the unannealed state have we obtained evidence for cross fusion between DSL and DPL vesicles.

Our results, therefore, may have implications for proposed effects of lateral phase separation on the permeability of a bilayer membrane¹⁰⁻¹². In particular an enhanced Na^+ permeation through membrane vesicles at the lipid phase transition temperature has been reported, and it was proposed that boundary regions between the solid and liquid crystalline phases facilitate this permeability increase¹¹. Whether these

boundaries between coexisting solid and liquid crystalline phases at T_c are similar to the metastable structural defects below T_c proposed here is now under investigation.

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Neurofilament disguise, destruction and discipline

NEUROFILAMENTS $\sim 100 \text{ \AA}$ in diameter appear in electron micrographs to be major constituents of most nerve cells, but their function is not clear and their shapes vary¹. In thin sections of brain they appear relatively thick and knobby¹; whereas in negative stain, squid neurofilaments appear smooth and cylindrical^{2,3}. Only neurofilaments from the squid giant axon³ and from calf brain^{4,5} have been isolated in quantities sufficient for analysis, but these differ in their solubility properties and subunit molecular weights. These inharmonious observations are partially clarified by the transformations reported here of neurofilaments from a new and nearly ideal source, the giant axon of a marine worm, *Myxicola infundibulum*.

Myxicola axoplasm can be extracted from the worm in about 10 s (ref. 6) (compare squid, 20 min; calf, 3 h), an important factor in view of the time-dependent changes reported here. *Myxicola* axoplasm is a highly structured gel consisting almost exclusively of neurofilaments^{6,7,17}. We have detected no microtubules. Electron microscopy (Fig. 1) indicates that *Myxicola* neurofilaments assume a variety of shapes when processed in different ways. Reasonable care has been taken to identify and control the pitfalls of electron microscope assay, such as sampling errors, and staining, drying and proteolytic artefacts.

In thin sections, *Myxicola* neurofilaments resemble most other neurofilaments¹, if fixed in the usual manner; that is, with glutaraldehyde, while inside an intact axon (Fig. 1a; diameters 70-280 Å). Finer fibrous arrays (Fig. 1b; diameters 15-120 Å), resembling microfilaments¹, appear throughout the axoplasm, if it is fixed after extraction from the axon. Though the fixatives used were identical, the environments of the proteins during fixation and the rates of fixation probably differ in Fig. 1a and b, because the membranes surrounding the axon act as a considerable diffusion barrier in Fig. 1a, but are absent in Fig. 1b. Figure 1, a and b

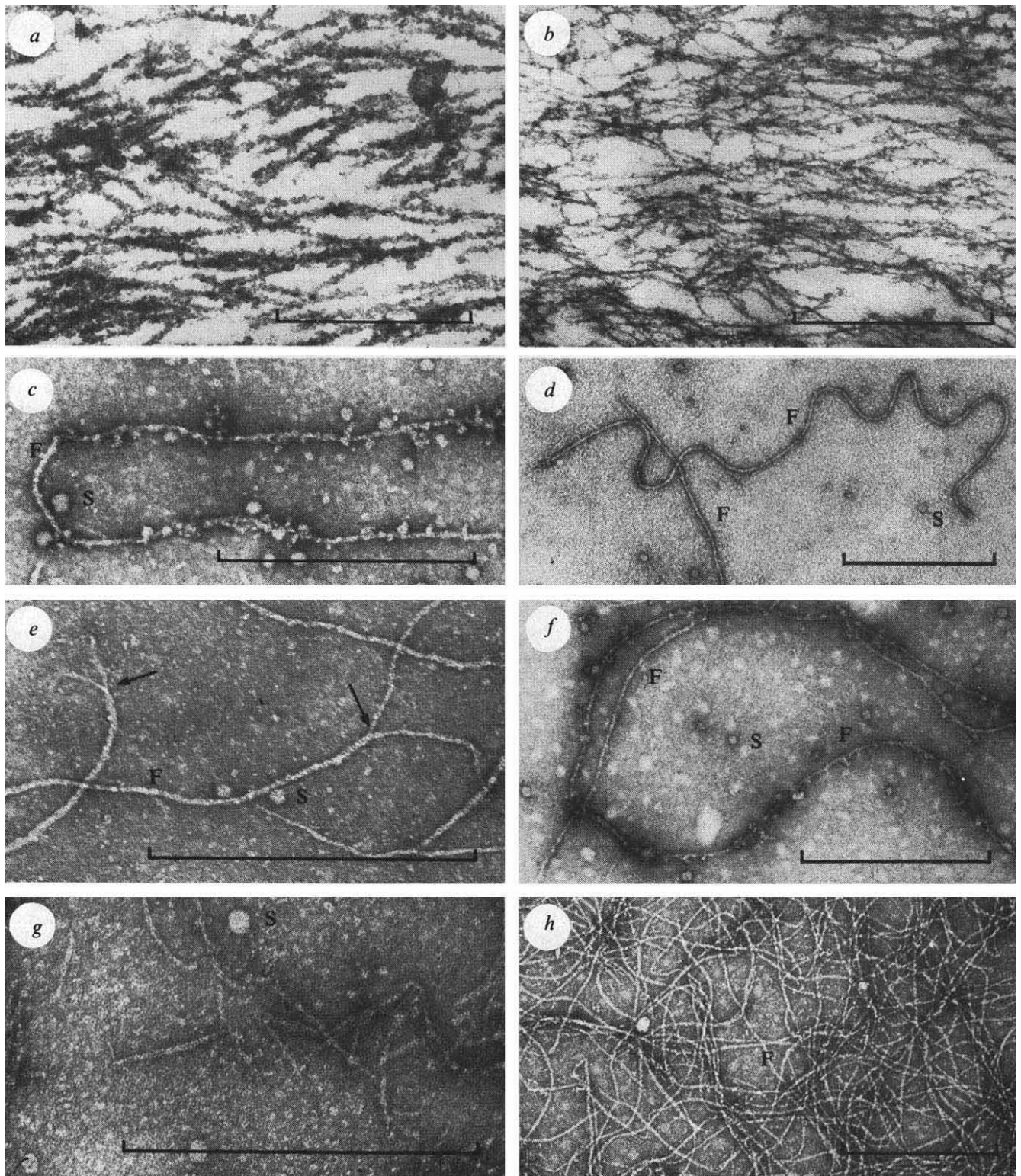


Fig. 1 Electron micrographs of *Myxicola* neurofilaments. *a, b*, Thin sections; *c-h*, negatively stained with 1% uranyl acetate on carbon-formar coated grids. *a*, Whole ventral cord fixed for 90 min (2% glutaraldehyde, 70 mM cacodylate, pH 7.2, adjusted to 1.01 osmolal with triple-strength artificial seawater), 23 °C. Postfixed with 1% OsO₄ with similarly adjusted tonicity and pH. Dehydrated in ethanol, embedded in Araldite, sectioned, and stained with uranyl acetate and lead citrate. Examined on a Siemens 1A at 80 kV. *b*, Extracted axoplasm, processed as in (*a*). *c*, Extracted axoplasm rinsed in buffer (20 mM cacodylate, pH 7.0) fixed 30 min in the above fixative, and homogenised in buffer. *d-f*, Extracted axoplasm homogenised (*d*) in buffer, (*e*) in distilled water, showing frayed filaments (→), and (*f*) in buffer containing 5% sucrose. *g*, Extracted axoplasm homogenised in buffer containing 0.5 M KCl; then rapidly diluted with 5 volumes of water, and applied to the grid. *h*, Reconstituted filaments, from the same fraction described in Fig. 2*f*. Calibration bar 0.5 μm. Abbreviations: F, ~ 70 Å diameter filament; S, ~ 280 Å diameter sphere.

indicates that the state of aggregation of the neurofilament proteins can be altered. Negatively-stained preparations (Fig. 1, *c-h*) point to the same conclusion.

Fixation (as for either Fig. 1*a* or *b*), followed by homogenisation and negative staining, yields filaments (F) with irregular, globular projections, as well as roughly spherical particles (S), which often appear attached to the filaments (Fig. 1*c*). If fixation is omitted, or occurs after homogenisation, most filaments have no projections, and detached spheres are numerous (Fig. 1*d*). Filaments are 72 ± 10 Å diameter (mean $\pm$ s.d.), and up to $10 \mu\text{m}$ long. Spheres are 280 ± 40 Å diameter. Both are proteins, since they are rapidly destroyed by Pronase. Filaments usually taper at their ends, but sometimes appear to untwist and fray into finer fibrous components (~ 15 – 50 Å in diameter, Fig. 1*e*, arrows). Sucrose and glycerol solutions often show projections and spheres attached to the filaments (Fig. 1*f*). High ionic strength solutions ($\mu\text{M} > 0.5$) reversibly dissociate both worm (Fig. 1*g*, *h*) and squid filaments^{2,3}, but not those from brain^{4,5}.

Of the several possible interpretations of the above data, we favour this: 70 Å filaments consist of a twisted array of fibrous, largely α -helical⁷ subunits, linked primarily by electrostatic bonds, since they are dissociated in solutions of high ionic strength. Non-covalently bonded to the 70 Å filaments are long branches (not easily distinguished from the filaments in Fig. 1*b*), which can coalesce with either the filament, to form the small projections seen in Fig. 1*a*, *c* and *f*, or with each other, to form the larger projections, and the attached and detached spheres seen in Fig. 1*c-h*.

Sodium dodecyl sulphate (SDS) gel electrophoresis of whole *Myxicola* axoplasm gives a different, and less complex, pattern of polypeptide chains than whole squid (*Loligo*

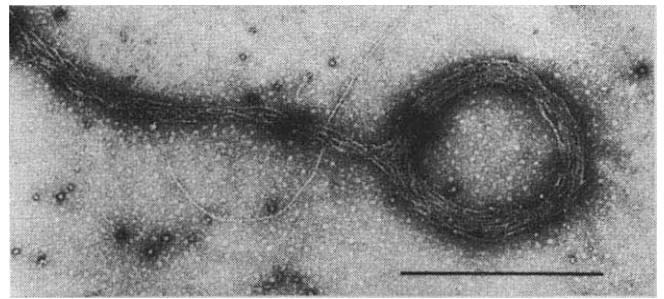


Fig. 3 A neurofilament ring, formed *in vitro* by *Myxicola* neurofilaments treated with cytochrome *c*. *Myxicola* axoplasm was homogenised in buffer (20 mM histidine, pH 7.0) and applied to a carbon-formvar coated grid. While still wet, the grid was rinsed with several drops of cytochrome *c* (Sigma, type IV, 0.2 mg ml⁻¹ in water, with 0.1% amyl alcohol), then inverted on a drop of 1% uranyl acetate, dried, and examined on a Phillips 300 at 60 kV. No such ordering is seen in the absence of cytochrome *c*. Calibration bar 1.0 μm .

forbesi) axoplasm (Fig. 2*a*, *b*). Whole *Myxicola* axoplasm has two dominant bands on SDS gels (Fig. 2*b*) corresponding to chain weights of $152,000 \pm 5,000$ (mean $\pm$ s.d., $n=19$; roughly 40% of total protein), and $160,000 \pm 4,000$ (roughly 10% of total protein). (Standards used for molecular weight determinations were: myosin, c-protein, cross-linked actin, tubulin, bovine serum albumin, lactate dehydrogenase, β -galactosidase and phosphorylase *a*.) Neither band comigrates with a dominant band from squid axoplasm.

Three observations suggest that *Myxicola* neurofilaments are composed of both the 152,000 and the 160,000 chains, together with lesser amounts of other chains, as shown in Fig. 2*c*, *d* and *f*. First, and most generally, electron microscopy indicates that neurofilaments account for the great majority of proteins in axoplasm, and these bands account for most of the protein on SDS gels. Second, and more specifically, repeatedly washed *Myxicola* neurofilaments (Fig. 2*d*) show bands similar to those of whole axoplasm, and in approximately their original proportions. Some heterogeneous and largely low molecular weight material is removed by this procedure (Fig. 2*e*). Third, neurofilaments reconstituted after dissociation in high salt concentrations show the same set of bands (Fig. 2*f*). We have not yet eliminated the possibility of contaminants either copurifying, or adsorbed to the neurofilament subunits; nor have we yet identified particular bands with each of the structural components of the neurofilament.

Calcium ions cause major changes in *Myxicola* axoplasm. Incubation with calcium for as little as 15 s causes a shift in the neurofilament gel pattern to lower molecular weight positions (Fig. 2*g-i*). Negatively-stained Ca^{2+} -treated filaments fray into fine fibres, then decompose further into ill-defined, flocculent clots. We interpret these events as the action of a Ca^{2+} -activated protease, present in whole axoplasm. Washing axoplasm (as for Fig. 2*d*) removes this protease, since washed filaments with added 10 mM Ca^{2+} are stable (as judged by SDS gels and electron microscopy), but alter as described above if the first wash supernatant (Fig. 2*e*) is then added. The protease can be inhibited by PCMB (parachloromercuribenzoate, 1 mM), or TPCK (L-1-tosylamide-2-phenylethylchloromethyl ketone, 1 mM), or TLCK (N- α -p-tosyl-L-lysine chloromethyl ketone HCl, 1 mM), but not by PMSF (phenylmethylsulphonyl fluoride, 1 mM). No digestion has been detected in the absence of added Ca^{2+} , for incubations up to 90 h.

Calcium ions (1–10 mM) also cause a macroscopic, rapid dispersal of whole *Myxicola* and squid⁹ axoplasm in water. PCMB (1 mM) inhibits this effect in *Myxicola*, so this seems to be caused by the Ca^{2+} -activated protease. Using SDS gels and electron microscopy, we have confirmed the presence of a similar PCMB- and EDTA-inhibited protease in squid

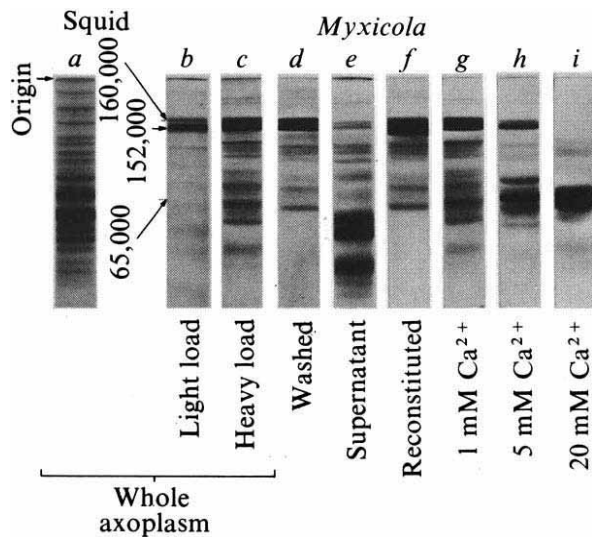


Fig. 2 SDS gel electrophoresis on 5% polyacrylamide of neurofilament preparations, essentially according to Weber and Osbourne¹⁷. *a*, Whole squid axoplasm; *b*, *c*, whole worm axoplasm; *c*, heavier loading of worm axoplasm, showing minor components. *d*, Washed neurofilaments, prepared by homogenising axoplasm in buffer (20 mM histidine, 0.1 mM PMSF, pH 7.0), centrifuging 2 h at 150,000*g*, resuspending the pellet, and repeating this cycle four more times. A low speed spin (20,000*g*; 15 min) was also included to remove particulate matter. The final pellet was used for (*d*), and the first supernatant is shown in (*e*). *f*, Reconstituted neurofilaments, prepared by homogenising axoplasm in buffer containing 0.75 M KCl, removing particulate matter (20,000*g*; 15 min), centrifuging (1.5 h; 250,000*g*), then dialysing the supernatant against buffer containing 0.1 M KCl, and sedimenting the reconstituted neurofilaments (2 h; 150,000*g*). *g*, *h* and *i*, Whole axoplasm after incubation (40 min, 20°C) with calcium ions. 20 mg axoplasm ml⁻¹ was homogenised in buffer and incubated with (*g*) 1 mM, (*h*) 5 mM and (*i*) 20 mM CaCl_2 , then boiled in 1% SDS, 1% 2-mercaptoethanol, to terminate digestion.

axoplasm¹⁰. A similar proteolytic activity may be involved in the Ca^{2+} -sensitive retrograde degeneration of vertebrate axons, in which a transition from filamentous to flocculent forms also occurs^{11,12}.

Cytochrome *c* causes the formation of remarkably ordered aggregates of *Myxicola* neurofilaments (Fig. 3). Fresh, or washed, or reconstituted neurofilaments align side by side to form neurofibril-like structures, which frequently terminate in rings. The rings are similar in size and shape to the neurofibrillar rings found *in vivo*, at synapses, and near cut axons^{6,13-15}. Rings lacking stems, figure eights and more complex forms are also synthesised *in vitro*, resembling the range of forms found *in vivo*¹³.

In summary, *Myxicola* neurofilaments resemble those of both vertebrates and invertebrates in their appearance in electron microscope sections, and in their neurofibrillar and ring-like assemblies. Different treatments, however, cause marked changes in *Myxicola* neurofilament appearance, which we tentatively identify with different states of aggregation of fibrous subunits. The protein molecule(s) involved have not yet been identified, but their component polypeptide chains give a simple and consistent pattern on SDS gels. The major chains (152,000 and 160,000 molecular weights) are rapidly cleaved by an endogenous, Ca^{2+} -activated protease, to give fragments roughly the size of the chains isolated from squid (70,000–90,000 (ref. 3)) and calf (~58,000 (ref. 4)) neurofilaments. But squid axoplasm has no major heavy chains which comigrate with those from *Myxicola*. Therefore present evidence suggests that neurofilaments from different species consist of different polypeptide chains, though possible proteolytic degradation of a common subunit cannot be ruled out.

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Persistent innervation of mammalian sympathetic neurones by native and foreign fibres

CONSIDERABLE evidence has been advanced to support the view that the reinnervation of adult mammalian autonomic

neurones is specific¹⁻⁴. Moreover, although foreign nerve fibres by themselves readily form synaptic connections with denervated ganglion cells⁵⁻¹¹, when native fibres are also present their terminals are thought to be preferred to foreign axon terminals, which are eventually rejected⁴. I have re-examined the ability of neurones in the mammalian superior cervical ganglion (SCG) to select between native and foreign nerve fibres during reinnervation using the technique of intracellular recording. An unexpected result was that many neurones were initially reinnervated by both native and foreign nerves, and retained their dual innervation for at least 10 months.

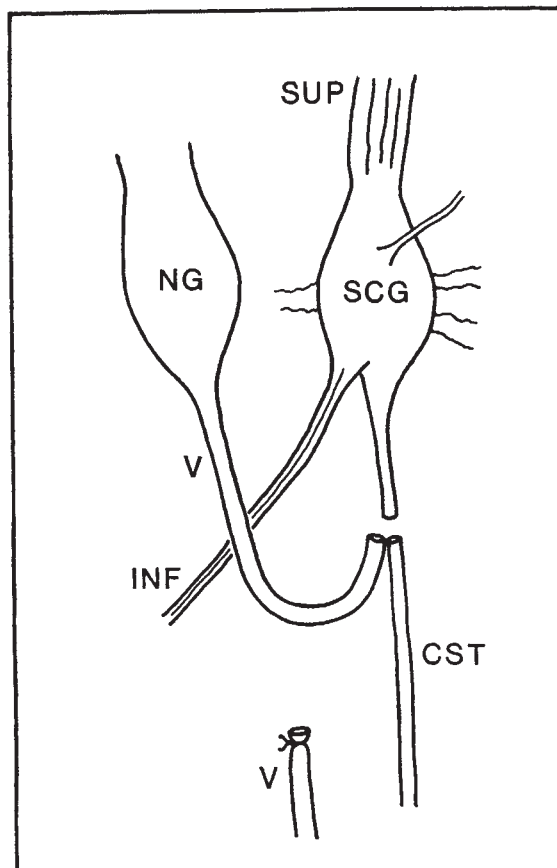


Fig. 1 Diagram of operative procedure (ventral view). NG, nodose ganglion; V, vagus nerve; SUP, superior postganglionic nerve bundle; INF, inferior postganglionic nerve bundle. Drawing not to scale.

The right cervical sympathetic trunk (CST) in 17 adult albino guinea pigs (200–400 g) was aseptically exposed under pentobarbital anaesthesia, and cut 3–5 mm below the lower pole of the SCG. The right vagus nerve was also cut about 2–3 mm caudal to this level, and its proximal end laid side by side with the proximal end of the CST (Fig. 1); the distal end of the vagus nerve was then ligated and the wound closed. After intervals ranging from 23 d to 10 months, the SCG was removed together with suitable lengths of the CST, the vagus nerve, and the superior postganglionic nerve, and placed in a perfusion chamber. In 14 animals, both the CST and the vagus nerve entered the neuroma which had formed at the nerve anastomosis; in three animals the vagus nerve did not enter the neuroma, and these ganglia were not considered further. The CST and the vagus nerve proximal to the anastomosis, and the superior postganglionic branch (Fig. 1), were taken into close fitting suction electrodes for stimulation and recording. Neurones within the ganglion were impaled with glass microelectrodes¹² and their responses to CST and vagal stimulation recorded.

By 23 d after the initial surgery, most impaled neurones

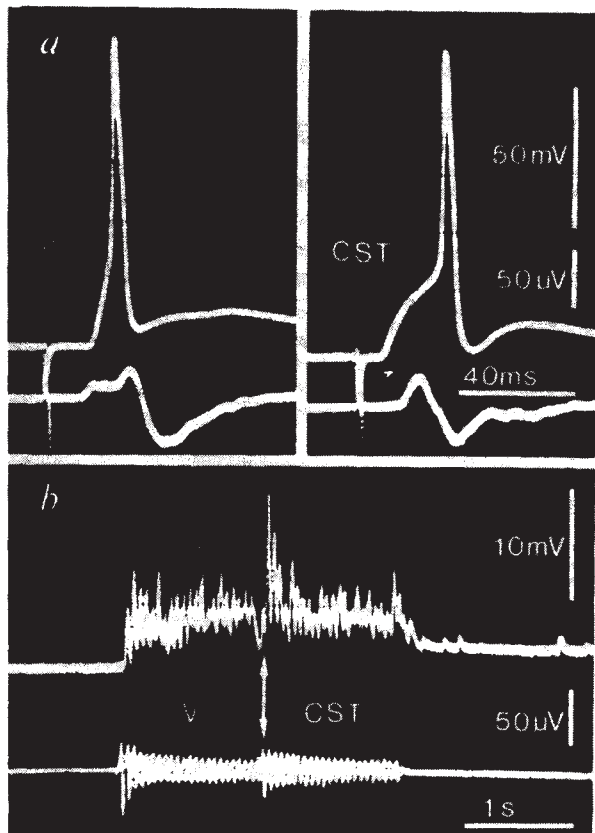


Fig. 2 *a*, Dually innervated neuron impaled 273 d after initial operative procedure. Upper traces show that supra-threshold responses are elicited following stimulation of both the native (CST) and vagus (V) nerves. Lower trace is an extracellular recording from the superior postganglionic nerve bundle. *b*, Upper trace shows that repetitive stimulation (20 s^{-1}) of the vagus nerve causes a depression of transmission in a dually innervated neuron impaled 291 d after initial operative procedure, but does not affect the response to subsequent stimulation of the CST (arrow). This effect can also be seen in the compound action potentials recorded from the superior postganglionic nerve bundle (lower trace). The intracellularly recorded effect was clearer when, as in this cell, the e.p.s.p.s elicited were subthreshold and thus not obscured by action potentials.

were found to be reinnervated. In about half the neurones excitatory postsynaptic potentials (e.p.s.p.s) were elicited after stimulation of only the CST or the vagus nerve; in the remainder, however, e.p.s.p.s were recorded in response to stimulation of both nerves. In a series of 101 neurones impaled in eight ganglia 23 d to 3 months after nerve section, synaptic potentials could be evoked in all but three cells; 60 neurones (59.4%) had e.p.s.p.s after both CST and vagal stimulation and, in many of these cells (28.3%), the response was above threshold for either nerve (Fig. 2*a*). That these neurones were influenced by two separate sets of nerve terminals was shown by summation of the e.p.s.p.s when the two nerves were stimulated at an interval adjusted so that the e.p.s.p.s elicited from both sources occurred simultaneously. Furthermore, depression of transmission caused by repetitive stimulation of one pathway did not depress transmission caused by stimulating the other nerve (Fig. 2*b*). Of the neurones which received innervation from a single source, about half were innervated by the CST, and half by the vagus nerve.

After 6–7 months, 19 out of 41 neurones (46.3%) impaled in two ganglia were found to be dually innervated, whereas after 8–10 months, 42 out of 78 neurones (53.8%) in four ganglia could be shown to receive endings from both sources. Thus little change occurred in the proportion of dually innervated cells over a 10 month period. There was also no obvious tendency for CST synapses to predominate

over vagal terminals: even after 8–10 months the portion of neurones having suprathreshold responses to both vagal and cervical trunk stimulation (Fig. 2*a*) was about one-third. Indeed, a greater number of neurones were innervated by vagal fibres than CST fibres after 8–10 months, although after 23 d to 3 months the number of cells innervated from each source was nearly equal.

These results should not be taken to indicate that connections within the SCG are not normally highly specific^{1–5}, or that ganglion cells may not have some ability to select correct synaptic connections in other circumstances. It may be, for example, that the large number of vagal fibres which grow into the ganglion overwhelm some more subtle selective mechanism. In the conditions of these experiments, however, mammalian sympathetic neurones show little or no preference for native or foreign fibres during reinnervation, and, over a period of 10 months, have no obvious tendency to reject inappropriate synaptic connections. This latter finding is similar to a recent study of dually innervated muscle fibres in mammals¹³ (see also ref. 14). Thus the adult mammalian nervous system seems less capable of selectivity during reinnervation than has previously been thought, and in this regard seems to be quite different from the nervous systems of lower vertebrates in which this ability is clearly present (see, for example, refs 15 and 16).

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Sex difference in ontogenesis of circadian adrenocortical rhythm in cortisone-primed rats

THE hormonal milieu in the neonatal period has profound influences on the morphological, biochemical and functional development of the brain^{1,2}. Krieger reported that the administration of corticosteroid (dexamethasone or hydrocortisone) to 2–4-d-old rats suppressed the circadian rhythm of plasma corticosterone at 30 d of age³, whereas treatment at 12–14 d of age did not affect the rhythmicity. We have investigated whether the suppression of corticosterone rhythm results in its permanent derangement throughout adult life or merely indicates its delayed appearance. After this paper was submitted, Krieger published the results of a similar study⁴.

Litters of Wistar rats were reared in a room with 12-h light and 12-h dark cycle (lights on 0600–1800). Within 72 h after birth, all pups were injected subcutaneously with either 0.5 mg of cortisone acetate (Upjohn Co., Kalamazoo, Michigan) or an equal volume of saline. Rats were weaned at day 27, group-housed by sex and treatment, and handled every day. Food and water were available *ad libitum*. On

Table 1 Effect of neonatal cortisone administration on morning and evening levels of plasma corticosterone

Treatment	Age (d)	Plasma corticosterone ($\mu\text{g dl}^{-1}$, mean $\pm$ s.e.)			
		0800	Male 2000	Female 0800	2000
Control	32	11.2 $\pm$ 0.9 (8)	33.1 $\pm$ 2.3*(8)	12.8 $\pm$ 1.1 (8)	36.2 $\pm$ 2.3*(8)
	42	10.1 $\pm$ 0.4 (6)	39.5 $\pm$ 2.8*(6)	13.8 $\pm$ 1.7 (6)	43.7 $\pm$ 2.0*(6)
	55	8.4 $\pm$ 1.5 (10)	38.4 $\pm$ 1.8*(10)	15.8 $\pm$ 1.4 (10)	47.5 $\pm$ 2.9*(10)
	95	15.9 $\pm$ 1.4 (5)	42.0 $\pm$ 3.4*(7)	16.1 $\pm$ 2.8 (6)	50.3 $\pm$ 5.1*(6)
Cortisone	32	12.2 $\pm$ 0.9 (8)	15.1 $\pm$ 1.0 (8)	14.0 $\pm$ 1.0 (8)	15.0 $\pm$ 1.4 (8)
	42	11.6 $\pm$ 0.9 (6)	13.3 $\pm$ 1.7 (6)	15.7 $\pm$ 1.4 (6)	24.3 $\pm$ 2.0*(6)
	55	9.7 $\pm$ 0.9 (10)	11.3 $\pm$ 1.4 (10)	16.1 $\pm$ 1.6 (10)	40.5 $\pm$ 2.5*(10)
		7.9 $\pm$ 1.3 (6)†	12.7 $\pm$ 1.4 (6)†	12.1 $\pm$ 0.5 (6)†	34.9 $\pm$ 2.7*(6)†
	95	11.9 $\pm$ 0.6 (5)	13.0 $\pm$ 1.0 (7)	—	—
		12.3 $\pm$ 1.4 (6)†	22.7 $\pm$ 0.3*(6)†	19.3 $\pm$ 3.2 (6)†	49.7 $\pm$ 3.5*(6)†
	130	6.8 $\pm$ 1.0 (8)	33.8 $\pm$ 2.6*(8)	16.3 $\pm$ 1.5 (8)	48.5 $\pm$ 4.3*(8)

* $P < 0.01$ against 0800 by Mann-Whitney U test.

†Data derived from the second series of experiments (see text).

Numbers of animals given in parentheses

days 32, 42, 55, 95 and 130, animals were decapitated at either 0800 or 2000 and trunk blood was collected for plasma corticosterone determination⁵. At each sampling time, the experimental groups were arranged so that each group represented a cross section of the litters available. A preliminary study indicated that these two time points gave the trough (0800) and the peak (2000) of plasma corticosterone rhythm in the condition used. On day 55, additional males were tested for plasma corticosterone response to ether stress at 1000. Animals were exposed to ether for 1.5 min and blood was obtained 15 min after the onset of stress⁶. In the other series of experiments, all animals were treated neonatally with cortisone and housed in the same way. On days 55 and 95, the profile of plasma corticosterone over a 24-h period was studied by blood sampling at 6-h intervals starting at 0800.

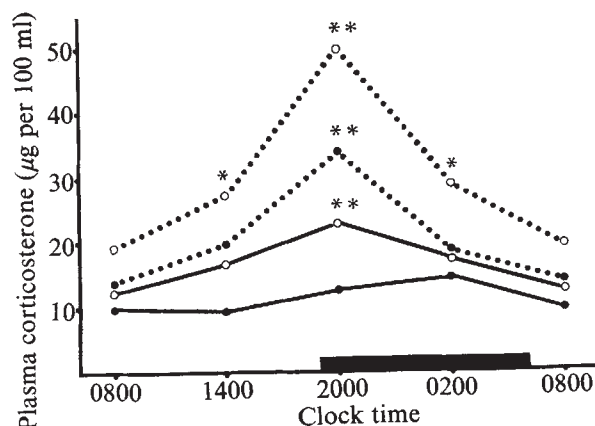


Fig. 1 Circadian variations of plasma corticosterone in cortisone-treated rats. Each data point represents the mean of six animals. —, Male rats; ·····, female rats; ●, 55 d of age; ○, 95 d of age. *, $P < 0.05$; **, $P < 0.01$, compared with 0800 value (Mann-Whitney U test). Dark period is shown as black horizontal bar.

The day-night variation of plasma corticosterone level was apparent from 32 d of age in the control rats of both sexes. Neonatal cortisone administration did not change the 0800 level significantly, but suppressed the rise of the 2000 level completely at 32 d of age in both sexes. At 42 d of age, however, the cortisone-treated female rats showed a small but definite nocturnal rise of plasma corticosterone. At 55 d of age, and thereafter, the adrenocortical rhythm was completely restored. In contrast, the effect of exposure to cortisone was more marked and lasted longer in the male rats. The plasma corticosterone rhythm was absent on day 55, equivocal or diminished on day 95 but ultimately established on day 130 (Table 1). No phase shift in circadian

rhythm was induced by the treatment (Fig. 1). The fact that the response of plasma corticosterone to ether stress was not impaired (Table 2) supports the concept that basal circadian rhythm and stress-induced change in the hypothalamo-pituitary-adrenal axis are regulated by somewhat different mechanisms⁷. Although corticosterone response to ether stress has been reported to have circadian variation⁸, we did not test whether neonatal cortisone treatment suppressed such rhythm of stress responsiveness.

Table 2 Response of plasma corticosterone to ether stress in 55-d-old male rats treated neonatally with cortisone

Treatment	Plasma corticosterone ($\mu\text{g dl}^{-1}$, mean $\pm$ s.e.)	
	Basal	Stress
Control	7.7 $\pm$ 1.0 (6)	50.4 $\pm$ 5.0*(6)
Cortisone	11.2 $\pm$ 1.5 (6)	47.4 $\pm$ 4.5*(6)

* $P < 0.01$ against basal by Mann-Whitney U test.

Numbers of rats given in parentheses.

Sex difference in the vulnerability of corticosterone rhythm to neonatal cortisone administration was rather unexpected. In Krieger's study⁴, the hydrocortisone-treated rats of both sexes, were reported to have 'normal' corticosterone rhythm at 80 d of age. Since data on earlier days are lacking, the results do not necessarily mean the same recovery course of corticosterone rhythm for both sexes. Furthermore, Krieger's figures show that the mean plasma corticosterone level at 2000 in the hydrocortisone-treated male rats was about half of that in the control male rats. The failure to find a significant difference between the two values might have resulted from the number of animals studied (only three rats at each time point) or a relatively large variation in the corticosterone level at 2000, as shown in our data on 95-d-old male rats treated with cortisone. The results of the two studies are not, therefore, mutually exclusive.

Concerning the sex difference of adrenocortical periodicity, Critchlow *et al.* have shown that a higher resting level and marked circadian fluctuation of plasma corticosterone are associated with the presence of mature ovary⁹. It has also been shown that a rather marked enhancement in the amplitude of circadian corticosterone rhythm takes place at puberty in the female, and that ovariectomy attenuates the change^{10,11}. Although adrenal-gonadal interactions are multiple and complex¹², a marked sex difference in the circadian rhythm of hypothalamic corticotrophin-releasing factor content and its phase shift by ovariectomy, reported by Hiroshige *et al.*¹³, provide good evidence that gonadal hormones can modulate the pituitary-adrenal periodicity through a central mechanism.

In rats and in many other species, sexual differentiation

of the brain is initiated in neonatal life. The male pattern is programmed by the presence, and female pattern by the absence, of androgen during a relatively short period of infancy known as the critical period¹⁴. This 'program' is stored for a while and expresses itself at puberty. It is therefore important to study whether the sex difference in the ontogenesis of plasma corticosterone periodicity observed in rats primed with cortisone in infancy is related to event(s) in early sexual differentiation or rather to factor(s) associated with the onset of puberty.

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Oestrogen-responsive human breast cancer in long term tissue culture

STUDIES of steroid hormone dependence of human breast cancer have been hampered by a lack of a suitable *in vitro* model system which can reproduce the responses of human breast cancer observed *in vivo*. We now report the characterisation of several human breast cancer cell lines, at least one of which shows marked dependence on oestrogens for growth. This oestrogen dependence seems to be similar in a variety of ways to that observed clinically *in vivo*.

MCF7 cells, a cloned line of human breast cancer, have been partially characterised¹. This line was found by a specific enzymatic assay in which the synthesis of lactose was confirmed by chromatography of the products (M.E.L. and B. V. Vonderhaar, unpublished), to synthesise α lactalbumin, and formed 7.05 pmol lactose per 30 min per μ g protein. Other breast cancer cell lines used—HT39, Kielty and G11—were derived from malignant effusions in patients with metastatic breast cancer. Under the electron microscope, these cell lines appear epithelioid with numerous microvilli, desmosomes, some rough endoplasmic reticulum and Golgi apparatus.

As physiological concentrations of steroid hormones are present in the serum containing media in which the cells are usually grown⁶ and as these endogenous hormones may obliterate responses to additional hormones, the serum was stripped of most endogenous steroid by repeated mixing with dextran-coated charcoal at 55 °C for 45 min⁷. Removal of oestradiol was monitored by the addition of a trace amount of tritiated oestradiol at the beginning of the procedure and after the decrease in radioactivity, which was reduced to less than 1% after successive charcoal treatments. Incubations of cells in various hormone combinations were carried out at least in triplicate. After various incubation periods the cells were pulsed for 1 h with labelled amino acids or nucleosides, collected and macromolecular synthesis measured⁸.

Table 1 shows the effects of oestradiol on DNA synthesis

in the MCF7 cell line. Oestradiol 10^{-8} M more than doubles nucleoside incorporation in the experiment shown. We noted reproducible increases in macromolecular synthesis with as little as 10^{-11} M oestradiol. If oestradiol (10^{-9} – 10^{-7} M) is added to cells in untreated serum no augmentation of macromolecular synthesis is noted. Oestradiol stimulation of these cells shows a clear-cut maximum at concentrations of 10^{-10} – 10^{-7} M oestradiol. The concentration of oestradiol which half saturates the oestradiol receptor *in vitro* closely corresponds to that concentration of oestradiol which half-maximally stimulates the cells, as determined by competitive protein-binding assay using dextran-coated charcoal (see below). Higher concentrations of oestradiol are less effective and concentrations of about 2×10^{-6} M clearly inhibit the cells below control levels (Table 1). Diethylstilboestrol, which stimulates these cells as effectively as oestradiol, also inhibits the cells at more than 5×10^{-7} M.

Table 1 Effects of oestradiol, diethylstilboestrol (DES) and Tamoxifen on DNA synthesis

Condition	Thymidine incorporation
Control	18 $\pm$ 4.3
Oestradiol 10^{-8} M	41 $\pm$ 5.2
Oestradiol 5×10^{-6} M	6 $\pm$ 2.6
DES 10^{-8} M	43 $\pm$ 3.1
DES 5×10^{-6} M	5 $\pm$ 1.7
Tamoxifen 10^{-7} M	4 $\pm$ 1.6
Tamoxifen 10^{-7} M + oestradiol 10^{-8} M	26 $\pm$ 4.8

Cells growing in log phase were collected and plated in quadruplicate at uniform density in Eagle's MEM supplemented with 10% foetal calf serum, and $2 \times$ glutamine. After 24 h the medium was replaced with MEM containing 2% serum from which endogenous steroids were removed by stirring for 45 min with dextran-coated charcoal at 55 °C twice. The medium was sterilised by filtration. Various hormones were added to the medium as $1,000 \times$ concentrates in ethanol as shown in Fig. 1. After 48 h 0.5μ Ci 3 H-thymidine (New England Nuclear, 40 Ci mmol⁻¹) was added to each dish. One hour later the dish was washed with phosphate-buffered saline twice and the cells collected after detachment with trypsin-EDTA solution. The cell buttons were suspended in ice water and sonicated in a Brownson sonicator for 4 s at the lowest setting. Aliquots were used for determination of protein by the method of Lowry¹³ and for TCA precipitation. Acid-insoluble counts were collected on 0.45 μ m Millipore filters and counted in a liquid scintillation counter (efficiency 35%). Values (d.p.m. $\times 10^{-3}$ per μ g protein per h) are averages of quadruplicate determinations ± 1 s.d.

This biphasic response to oestrogen suggests that the same population of cells which is stimulated by oestradiol at lower concentrations of oestrogen may be inhibited at higher concentrations. As such concentrations are readily achieved in patients with metastatic breast cancer treated with large doses of oestrogen, our data suggest that this regimen may be effective because the tumour itself is directly inhibited by the oestrogen rather than by an indirect effect of the oestrogen on some other trophic hormone. This is an attractive explanation for the seemingly paradoxical stimulation of some tumours by oestrogen at physiological levels and tumour regression seen at higher doses of administered oestrogens⁹. To demonstrate more clearly the oestrogen dependence of these cells, we used the anti-oestrogen ICI46,474 (Tamoxifen), which is a substituted hexoestrol derivative¹⁰. Figure 1 shows that 10^{-7} M Tamoxifen depressed DNA synthesis below control levels; this effect could be overcome by simultaneous addition of 10^{-8} M oestradiol. Hundredfold lower concentrations of oestradiol were sufficient to overcome Tamoxifen inhibition of macromolecular synthesis. Similar effects were noted when incorporation of 14 C-leucine into protein was measured.

Figure 1 shows the time course of the response to both oestradiol and Tamoxifen. At varying times after the addition of Tamoxifen to the cells, isotopically labelled pre-

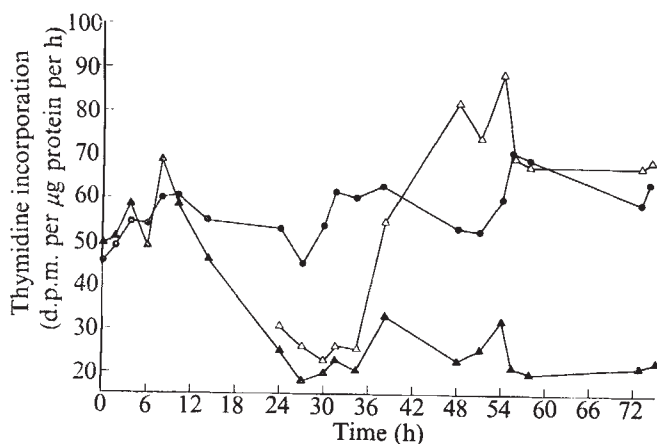


Fig. 1 Kinetics of Tamoxifen inhibition and oestradiol stimulation of human breast cancer *in vitro*. Cell plating and collection, see Table 1. ●, Control cells; ▲, 10^{-7} M Tamoxifen-treated cells. After 24 h, 10^{-8} M oestradiol was added to an additional set of dishes without removing the Tamoxifen (△). s.d. for each point was usually less than 10%.

cursors were added for 1 h for determination of net synthetic rate at that time point. By about 10 h, 10^{-7} M Tamoxifen begins to strongly inhibit macromolecular synthesis which falls to about one-third of control levels by 24 h and remains at this level throughout the duration of the experiment. If at 24 h, 10^{-8} M oestradiol is added to some of the Tamoxifen-containing dishes without removing the Tamoxifen, the inhibition is shown to be completely reversible with recovery beginning about 10 h after addition of the oestradiol. Similar changes are noted when protein synthetic rates are measured. We have observed a rise in thymidine incorporation rates to values which exceed control levels for several hours during the early period of oestrogen stimulation of Tamoxifen-inhibited cells. This suggests that some degree of synchronisation of the cells may have occurred during the incubation in Tamoxifen alone, with a larger cohort of cells entering the DNA synthetic phase of the cell cycle after oestradiol rescue. If a cloned line of the hormone-responsive MCF7 cells are left in Tamoxifen alone, most cells begin to round up, detach from the surface and die after about 4 d although occasional colonies of 'normal' Tamoxifen-resistant and possibly hormone-independent cells are sometimes noted. The phenomenon of Tamoxifen killing is invariably reversible if oestradiol is added to the medium by 48 h even though the anti-oestrogen remains in the medium.

We have duplicated these experiments in serum-free conditions. In spite of the total lack of oestrogen in the medium, Tamoxifen still inhibits the cells below control levels. The possibility that Tamoxifen-oestrogen receptor complexes are binding to chromatin sites leading to decreased transcription of regulatory RNA segments below control levels is under investigation.

That Tamoxifen is inhibiting these cells primarily by interfering with the trophic effects of oestrogen is suggested by three lines of reasoning. First, Tamoxifen inhibition is reversible by the addition of oestradiol. Simultaneous addition of Tamoxifen and oestradiol leads to stimulation if the Tamoxifen is less than 2 logs higher in concentration than oestradiol. Second, in other experiments, Tamoxifen had no effect on cells in culture not responsive to oestradiol, such as hepatoma tissue culture cells, HeLa cells, or the HT39, Kielty and G11 breast lines. Third, Tamoxifen is capable of binding to the same high affinity oestrogen receptor molecules found in the cytoplasm of these cells. Using a dextran-coated charcoal assay¹¹ one of these cell lines, MCF7, was shown to contain about 70 femtomol oestradiol receptor per mg cytoplasmic protein with an apparent K_d of about 5×10^{-11} M, a value in reasonable agreement with

a previously published value¹². Tamoxifen binds to this receptor with about a 1,000-fold lower affinity than oestradiol, explaining in part the ability of 10^{-8} M oestradiol to stimulate the cells in the presence of 10^{-7} M Tamoxifen. In fact, the dose-response curve of the inhibition of these cells by Tamoxifen closely approximates the displacement curve of oestradiol from its cytoplasmic receptor by Tamoxifen.

The MCF7 line has been maintained in continuous tissue culture for 2 yr but still demonstrates remarkable oestrogen dependence. The potential value of a hormone-dependent human breast cancer in long term tissue culture for the study of the mechanism(s) by which steroid hormones exert their trophic effects is significant, particularly in view of the likelihood of obtaining regulatory variants or mutants which are hormone independent. In addition these cells may prove useful in the analysis of oestrogen action in non-malignant tissue, as oestrogen-responsive cell systems in continuous tissue culture have not been widely reported.

The Kielty, G11, and HT39 lines are not stimulated by 10^{-8} M oestradiol nor are they inhibited by Tamoxifen. They are, however, killed by 10^{-5} M oestradiol. The specificity of this response is being investigated.

In results to be reported elsewhere, two other human breast cell lines, Evsa T and Evsa E developed in our laboratory, both respond to 10^{-8} M oestradiol with the accumulation of α lactalbumin without a significant increase in general macromolecular synthesis¹.

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Non-encephalitogenic antigen-induced suppression and reversal of allergic encephalomyelitis

EXPERIMENTAL allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system¹ induced in experimental animals by the myelin basic protein (BP)² or specific peptide regions derived from the BP³ emulsified with Freund's complete adjuvant (FCA).

Previous studies have shown that the encephalitogenic tryptophan region of BP, H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-OH (peptide S3) induces delayed type hypersensitivity (DTH) specific for peptide S3 and BP (ref. 4). The DTH determinant was separated from that which produces disease and shown to overlap the C-terminal sequence of peptide S3

Table 1 Suppression and treatment of allergic encephalomyelitis with non-encephalitogenic peptide S42

Group no.	Challenging antigen (μ g per FCA)	Treatment FIA	Clinical EAE	Reversal clinical EAE	Histological EAE
1	25 μ g S42	None	0/15	None	0/15
2	100 μ g S42	None	0/10	None	0/15
3	5,000 μ g S42	None	0/10	None	0/10
4	100 μ g BP	1 mg S42	2/4	2/4	2/4
5	100 μ g BP	FIA	4/4	0/4	4/4
6	150 μ g BP	2.5 mg S42	5/5	5/5	2/5
7	150 μ g BP	FIA	5/5	0/5	5/5
8	150 μ g BP	saline	6/6	0/6	6/6
9	150 μ g BP	4.0 mg S42	7/7	6/7	4/7
10	150 μ g BP	1 mg BP	4/4	2/4	3/4
11	150 μ g BP	FIA	7/7	0/7	7/7

The guinea pigs were challenged with Freund's complete adjuvant (FCA) emulsion of either peptide S42 or bovine basic protein. Groups 1, 2 and 3 received no treatment. For groups 4 and 5, the suppressive treatment in Freund's incomplete adjuvant (FIA) was started on day 8, before the appearance of clinical signs of experimental allergic encephalomyelitis (EAE) and was continued through day 17. For groups 6, 7 and 8, the treatment began the day hind leg paralysis was observed and was continued for 10 consecutive days. For groups 9, 10 and 11, the treatment was started two days after hind leg paralysis was observed and was continued for 10 consecutive days. Surviving animals were killed on day 32 for histological examination of the brain and spinal cord tissue.

(ref. 5). Further, the immunological response of sensitised animals to the EAE or to the DTH determinant could not be distinguished clinically or histologically from similar responses to the intact BP molecule. We have shown^{6,7} that the encephalitogenicity of the tryptophan region is destroyed by amino acid substitutions or deletions from the sequence of peptide S3, but this treatment did not abolish the DTH properties of the resulting antigens. Deletion of the Gly-Ala-Glu-Gly sequence gives rise to peptide S17, H-Phe-Ser-Trp-Gln-Lys-OH, previously shown to induce and elicit DTH responses similar to those obtained for peptide S3. Further, animals sensitised to peptide S3, S17 or the BP exhibit DTH responses which were elicited by either of the three antigens⁷. These results indicate that the non-encephalitogenic antigen is recognised by cells sensitised to the EAE-inducing antigen and thus may be useful in the treatment of this cell-mediated disease. Peptide S42 was prepared by the Merrifield solid phase synthesis technique⁸ to contain four linearly spaced repeating units of S17. Approximately 700 mg of peptide S42 was prepared at one time. Based on 0.25 mEq glycine per gram resin, a recovery of 40–60% was calculated. The purity of peptide S42 was established by chromatography and high voltage and polyacrylamide disc gel electrophoresis⁹. Amino acid analysis¹⁰ of purified peptide S42, H-(Phe-Ser-Trp-Gln-Lys)₄-Gly-OH, gave whole integers of expected residues, including 4 mol tryptophan per mol peptide.

The results of experiments designed to study the efficacy of non-encephalitogenic peptide S42 in inhibiting the development (suppression) and in reversing (treatment) signs of EAE are summarised in Table 1. Daily subcutaneous injections of 1 mg peptide S42 starting on day 8 after challenge, significantly reduced the incidence of disease compared with Freund's incomplete adjuvant (FIA) treated controls (compare groups 4 and 5). Daily treatment of animals in group 6 with 2.5 mg peptide S42 for 10 consecutive days was sufficient to reverse tremor and hind leg paralysis in addition to lethargy and weight loss in the five treated animals. Histological examination of the brain and spinal cord tissues on day 32 after challenge revealed the presence of EAE lesions in 2 out of 5 of the animals in spite of the complete reversal of the clinical signs of EAE. Control guinea pigs similarly treated with FIA or saline (groups 7 and 8) died between days 10 and 14 or were killed because of severe paralysis.

Peptide S42-induced reversal of clinical signs of EAE was further studied in an advanced stage of the disease. The treatment for guinea pigs in group 9 was initiated on the second day after the onset of hind leg paralysis. Within 4–6 treatment days, paralysed animals regained the use of their hind legs and were able to run about the cage; however, one of seven guinea pigs died after the second treatment without evidence of improvement in his disease state. In contrast, treatment of

group 10 with 1 mg bovine BP was partially successful in reversing the disease at this late stage of development. None of the FIA-treated controls, group 11, recovered from EAE.

The mechanism of suppression and reversal of disease cannot be elucidated from this study. The primary event of the immune response requires the combination of antigen with receptors on the surface of reactive cells¹¹. Cell surface receptors in BP-sensitised animals are specific and recognise the encephalitogenic protein, the tryptophan region and analogous antigens such as peptide S42. This recognition may be described as antigen-induced paralysis of encephalitogenic cells leading to complete reversal of disease. Compared with the effect of anti-lymphocyte serum and cyclophosphamide^{12,13}, peptide S42 did not induce transient or prolonged lymphopenia, and the treated animals remained immunoresponsive during and after prolonged treatment with peptide S42. Repeated immunisation with peptide S42 failed to induce the formation of antibodies which reacted with BP or with peptide S42.

Studies have shown that intact BP injected before or shortly after the administration of an encephalitogenic dose of the BP reduces the incidence of EAE^{14,17}. The use of the BP for treatment, found to be effective in reversing signs of EAE in monkeys, is predicated on uninterrupted administration of the antigen during the critical period of disease¹⁷. The possibility of *de novo* induction of EAE with the administration of large doses of the encephalitogen should be recognised, however¹⁸. A synthetic random amino acid copolymer (cop 1) has been used to suppress disease development in guinea pigs¹⁹. Unlike peptide S42, cop 1 induces the formation of humoral antibodies. Its amino acid sequence is unknown and lacks phenylalanine, serine, tryptophan and glutamine residues known to be essential in the linear sequence necessary for maximum recognition by the EAE inducing cells.

The use of peptide S42 is advantageous in view of its non-encephalitogenic properties and its ability to interact specifically with disease-inducing cells and thus to inhibit the development of EAE and reverse a full-blown stage of disease.

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Isolation of infectious C-type oncornavirus from human leukaemic bone marrow cells

C-TYPE ONCORNAVIRUSES are associated with neoplasms of the haemopoietic organs in several vertebrate species¹. Molecular hybridisation studies have revealed the presence of an RNA species in human leukaemias that has some homology with animal C-type oncornaviruses^{2,3}. In addition, leukaemic cells contain a reverse transcriptase that proves to be serologically closely related to the enzyme from the woolly monkey C-type oncornavirus⁴. Several claims have been made for the presence of C-type particles in malignant cells or plasma of leukaemic

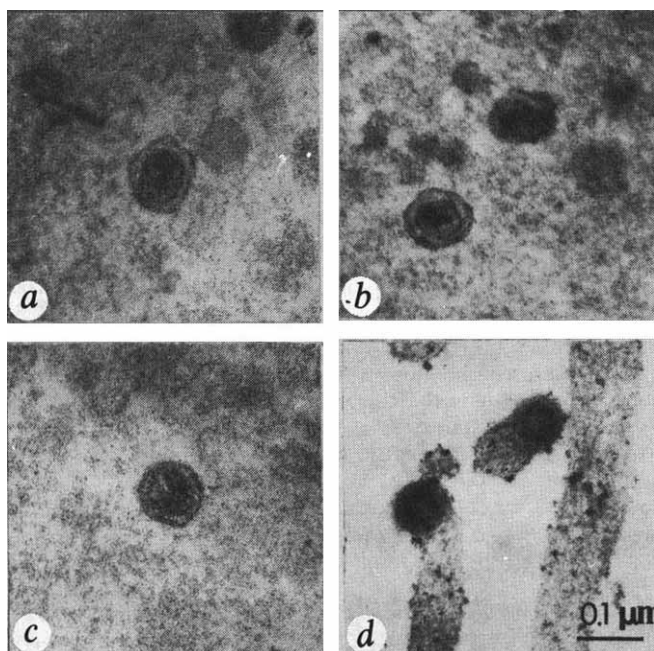


Fig. 1 C-type particles isolated from supernatants of culture fluids. Cleared fluids were spun in cellulose nitrate tubes at 50,000 r.p.m. for 45 min in a Beckman 50 Ti rotor. In the tubes, Millipore membrane filter disks (type 5 VSWP), pore size 25 nm, were positioned on top of supports made of Epon embedding medium. The fluid was mixed with sodium cacodylate-buffered 2% stabilised glutaraldehyde before the run. After the run, the disks were cut into thin oblong strips and immersed in phosphate-buffered 1% osmium tetroxide for 60 min (4 °C). They were then dehydrated in a graded series of isopropanol, treated with toluene, and flat embedded in Epon. Ultrathin sections were made and their contrast enhanced with an aqueous solution of manylacetate and lead citrate. They were examined in a Philips 300 electron microscope. *a*, Particle from phytohaemagglutinin-stimulated bone marrow cells. *b*, Particles from infected human embryonic fibroblasts. *c*, Particle from infected HEK cells. *d*, Budding particles from infected FB289.

patients (for review, see ref. 5). There have also been reports of release of such virus particles in tissue culture lines of leukaemic cells^{6,7}.

We report here the isolation of a C-type oncornavirus from a 4-yr-old patient with a lymphosarcoma which had progressed to a state of lymphoblastic leukaemia. Bone marrow cells were cultured in liquid suspension in the presence of phytohaemagglutinin (10 µl ml⁻¹, Burroughs, Wellcome, Lot No. 9268). After 3 d culture, the supernatant was examined for the presence of C-type particles using the Millipore filter technique⁸ and was found to be positive (Fig. 1).

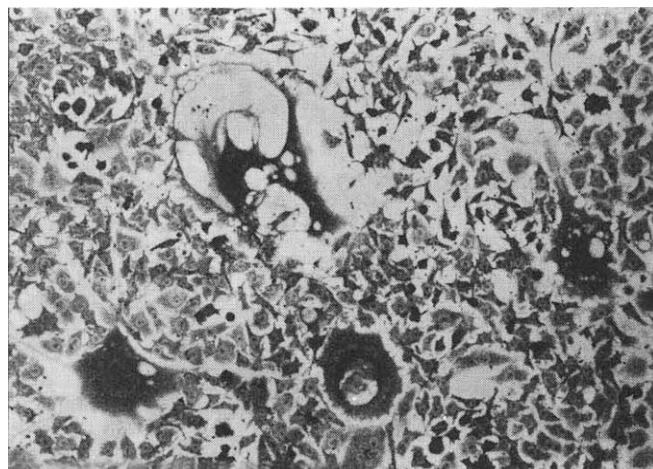


Fig. 2 XC cells with syncytia formed after cocultivation with human leukaemic bone marrow cells. XC cells were grown in the same medium as the bone marrow cells. Cells were grown in Dulbecco's modified MEM supplemented with 2 mM glutamine, 0.2 mM asparagine, 20% of a mixture of equal volumes of foetal bovine serum, normal horse serum, and tryptose phosphate broth containing 100 IU ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. One day after seeding of 2.10⁵ XC cells in 3 ml medium in 50 mm plastic Petri dishes (Greiner, Nürtingen), human bone marrow cells were added in 3 ml medium after removal of the initial medium. Medium was refreshed 3 d later and the dishes were stained with May-Grunwald Giemsa after 1 more d (×105).

Bone marrow cells were seeded on top of a 24-h culture of XC cells. Four days later, conspicuous syncytia were found which were similar to those reported to occur after cocultivation of XC cells and cells infected with a murine leukaemia virus⁹ (Fig. 2 and Table 1). In control XC cultures, some small syncytia which contained only a few nuclei were found.

The same cytopathogenic effect was found when fresh leukaemic bone marrow cells were cocultivated directly with XC cells. The production of syncytia persisted on further passage, after trypsinisation of the XC cells which had been in contact with the leukaemic cells.

Cocultivation of XC cells with bone marrow cells from four normal donors, two patients with acute myeloid leukaemia, two with chronic myeloid leukaemia, one with chronic lymphatic leukaemia, one with aplasia and one with secondary polycythaemia vera did not produce syncytia.

The XC cultures which showed a positive cytopathogenic effect released C-type particles, as revealed by electron microscopy (Table 1). Control XC cultures or mixed cultures of XC with normal bone marrow cells were repeatedly negative.

Secondary cultures of human embryonic kidney (HEK) cells were cocultivated with irradiated (9,000 rad X rays) XC cells in their second passage after having been in contact with leukaemic cells. HEK cells proved to replicate the human virus as detected by electron microscopy and the reverse XC cell test¹⁰. Control experiments with irradiated untreated XC cells did not yield detectable virus. The human embryonic fibroblast line (FB289) exposed to cell-free supernatants of infected HEK cells also replicates the virus.

Table 1 Detection of C-type oncornavirus by mixed cytopathogenicity test with XC cells and electron microscopic examination of culture fluids

Cocultivation of XC cells with:	No. of bone marrow cells plated	No. of syncytia per dish*	Electron microscopy
None	—	17†	—
BALB/c mouse BM infected with RLV	1.0×10^5	1,284	+
Normal human BM	1.0×10^5	10†	—
	5.0×10^5	18†	—
	1.0×10^6	16†	—
Leukaemic BM, stimulated by phytohaemagglutinin	1.0×10^5	471	+
Leukaemic BM, unstimulated	1.0×10^5	393	+
	2.5×10^5	738	—
	5.0×10^5	901	—
XC + leukaemic BM, 2nd passage	1.0×10^5	520	+
3rd passage	1.0×10^5	465	—
4th passage	1.0×10^5	606	+
XC + normal BM, 3rd passage	1.0×10^5	15†	—
HEK + irradiated XC (+ leukaemic BM), 2nd passage	1.0×10^5	352	+
HEK + irradiated XC	1.0×10^5	18†	—
HEK	1.0×10^5	14†	—
FB289 infected with supernatant from infected HEK cells	1.0×10^5	240	+
FB289	1.0×10^5	13	—

* Each experiment was done in quadruplicate. Petri dishes were coded and syncytia counted blindly and independently by two researchers using an inverted microscope at a magnification of $\times 75$.

† Only small syncytia with less than four nuclei.

BM, Bone marrow.

Indirect immunofluorescence tests were carried out on acetone-fixed cultured cells to assess the serological relationship of this putative human virus isolate to other mammalian C-type oncornaviruses, but also to exclude the possibility of laboratory contamination. At the time when the virus was isolated from the child the only C-type viruses used in our laboratories were murine leukaemia viruses (MuLVs). For that reason several antisera to murine C-type viruses were tested on these cultures (Table 2). Polyvalent rat antiserum to Rauscher murine leukaemia virus (RLV) numbers 45 and 76 have been used to study the presence of C-type oncornaviral antigens in human bone tumour cultures¹¹. Their specificity has therefore been extensively tested. They show a broad group-specific reactivity with various ecotropic and xenotropic mouse viruses, endpoint ranging from 1:1,280 to 1:10,240. Antiserum No. 45 not only reacts with an antigen in human bone tumour cultures which has been demonstrated to be specific for C-type virus¹¹, but

also weakly with the virus isolated from the leukaemic child and passaged in several kinds of cells. A goat antiserum to an endogenous C-type oncornavirus isolated from the BALB/c mouse mammary tumour cell line EMT6 (ref. 12) gives similar reactions. The strongest antiserum to RLV (76), however, does not react with either the human bone tumour cultures or the new virus isolated from the leukaemic child. This finding strongly indicates that the weak but positive reaction with two antisera is not due to contamination of the cultures with a murine virus but to an interspecies antigenic determinant not detected by antiserum No. 76.

A goat antiserum to the purified p27 antigen of the simian sarcoma virus (SSV)¹³ gives a good reaction with cultures harbouring the primate virus, although the acetone-fixed slides have been kept for 18 months at -20°C . The weak reaction with the new virus isolate is probably not only due to poor replication of the virus and in turn to decreased production of p27 in the cytoplasm, but also to an antigenic dissimilarity between the simian and the human virus.

Another source of contamination may be XC cells. Avian Rous sarcoma virus carried in XC cells does not replicate in human cells and is antigenically completely unrelated to mammalian viruses¹⁴. Control XC cultures proved to be repeatedly negative with all antisera. The cocultivation procedure may have activated an endogenous rat virus. Rat antiserum No. 76, however, reacts with endogenous viruses activated in XC cells by 5-bromodeoxyuridine ($20\text{ }\mu\text{g ml}^{-1}$) and 2% dimethylsulphoxide¹⁵, but not with XC cells cocultivated with the leukaemic cells. Goat antiserum to E6-MuLV shows quite the opposite pattern, indicating that contamination with endogenous rat virus may be excluded.

A second isolation was made 1 month later from the same patient, while in remission, by cocultivating the buffy coat of the peripheral blood with human embryonic fibroblasts FB289. Within 2 weeks virus was demonstrable by electron microscopy, XC plaque test and immunofluorescence. In the latter, rat serum No. 76 again gave no reaction, whereas goat anti-E6-MuLV was positive (1:40). Our conclusion is that we have twice isolated a C-type oncornavirus from a leukaemic child. The virus can replicate in human embryonic cells and in rat XC cells, in which it has a cytopathogenic effect. Possible laboratory contamination with animal oncornaviruses may be excluded. Biochemical studies of this isolate are required to assess in more detail its relationship to animal viruses and to determine whether it is exogenous or endogenous to human cells.

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Table 2 Immunofluorescence endpoint titration of cell cultures with antisera to animal C-type oncornaviruses*

Cell lines	Antisera			
	Rat anti-RLV 45	Rat anti-RLV 76	Goat anti-E6-MuLV	Goat anti-SSV1 p27
BALB/c 3T3 control	—	—	—	—
BALB/c 3T3 + RLV	2,560	5,120	2,560	20
XC control	—	—	—	—
XC + leukaemic bone marrow, 2nd passage	40	—	20	40
XC + 5-bromodeoxyuridine + dimethylsulphoxide	160	80	—	40
HEK control	—	—	ND	ND
HEK + irradiated XC (+ leukaemic BM)	40	—	ND	ND
HEK + irradiated XC	—	—	ND	ND
FB289	ND	—	—	—
FB289 + supernatant infected HEK cells	ND	—	40	20
Woolly monkey fibrosarcoma	320	80	80	320
Human adult fibroblast 1, 17th passage	—	—	—	—
Human chondrosarcoma, 15th passage	80	—	80	10

* Twelve spots on acetone-fixed slides were used for incubation. Nine of the wells were filled with different dilutions of the antiviral serum. Twofold dilutions were made in PBS, beginning at 1:10. One well was used for normal serum, one for only the fluorescein isothiocyanate (FITC)-conjugated anti-IgG serum and one for only PBS.

ND, Not determined. As two different FITC-conjugated antisera to goat IgG reacted with HEK cells the tests with goat antiviral sera were not carried out on HEK cells.

Department, Antoni van Leeuwenhoek Hospital, Amsterdam, and p27 antigen by Dr S. T. August, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, New York.

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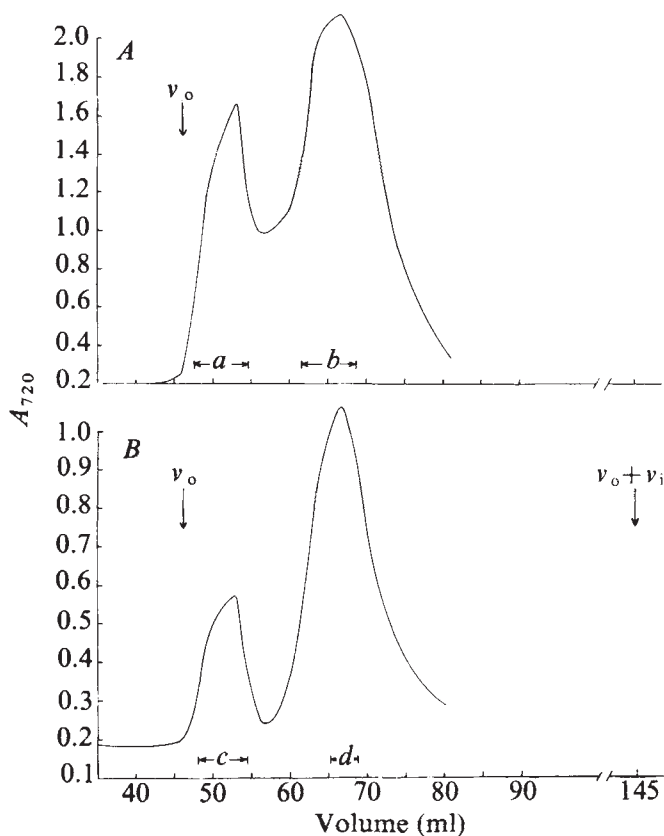


Fig. 1 Profile of Sepharose 4B agarose filtration of the 38-52% ammonium sulphate fraction of platelet actomyosin before (A) and after (B) phosphorylation. For both samples, a 1.65 ml aliquot containing 7.5 mg protein was applied to a 1.5×88 cm column equilibrated and eluted at 21 ml h^{-1} with 0.5 M KCl 15 mM Tris-HCl (pH 7.5), and 1 mM EDTA and 2.5 mM dithiothreitol. Samples (3.5 ml) were collected of which 0.05 ml was used for ATPase assay (shown above). Ordinate indicates A_{720} of the ATPase assay (control sample was incubated for a longer period of time than the phosphorylated sample). Void volume and salt boundary are indicated (V_0 and $V_0 + V_i$). Fractions were pooled as indicated for SDS-polyacrylamide gel electrophoresis (Fig. 2) and ATPase assays (Table 1). Protein concentration of pooled fractions was 0.2-0.3 mg ml^{-1} .

Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity

HUMAN platelet myosin, which is similar to other myosins isolated from non-muscle cells, has a molecular weight of 460,000 and is composed of two heavy chains (200,000) and two different light chains (20,000 and 15,000)¹⁻³. The 20,000 light chain can be phosphorylated by a kinase endogenous to human platelets³. This enzyme, which has been isolated and partially purified⁴, transfers ^{32}P from $\gamma\text{-}^{32}\text{P}\text{-ATP}$ to the 20,000 light chain of platelet myosin in the presence of Mg^{2+} . So far, the biological significance of this phosphorylation has been unknown, but we report here that phosphorylation of platelet myosin results in an increase in the actin-activated myosin ATPase activity measured at low ionic strength. Dephosphorylation of phosphorylated myosin results in a decrease in the actin-activated ATPase activity.

A 38-52% ammonium sulphate fraction of platelet actomyosin, containing the platelet myosin light chain kinase, incorporates 0.8-1.0 mol P_i per mol 20,000 light chain³. To study the effect of phosphorylation, the unphosphorylated ammonium sulphate fraction was divided in half. One half was phosphorylated with 3 mM MgCl_2 and 0.2 mM ATP and incubation at room temperature, pH 7.5 for 30 min. The control half of the sample was treated in the same manner but without ATP or MgCl_2 . Both the control and the phosphorylated myosin were then chromatographed on columns of Sepharose 4B (Fig. 1). In the case of the phosphorylated sample, agarose filtration terminated phosphorylation.

Two peaks of $\text{K}^+\text{-EDTA}$ -activated ATPase activity were eluted for each sample. As shown in Fig. 2, the first peak of ATPase activity is caused by platelet actomyosin and the second peak by platelet myosin alone. Representative fractions from each peak were analysed for actin-activated myosin ATPase activity measured in the presence of Mg^{2+} at low ionic strength and $\text{K}^+\text{-EDTA}$ - and Ca^{2+} -activated myosin ATPase activity assayed at high ionic strength.

Table 1 shows the effect of phosphorylation on myosin ATPase activity. The low ionic strength Mg^{2+} ATPase activity

of phosphorylated myosin is 0.17 $\mu\text{mol P}_i$ per mg protein per min after addition of rabbit skeletal muscle actin. Control (non-phosphorylated) myosin has a significantly lower specific activity (0.029) after the addition of the same amount of rabbit skeletal muscle actin. $\text{K}^+\text{-EDTA}$ - and Ca^{2+} -activated ATPase activities, measured at high ionic strength, are essentially the same for phosphorylated and control myosin.

The enzymatic activities for the initial peaks (actomyosin) from Sepharose chromatography are also shown in Table 1. Again, the actin-activated ATPase activity for phosphorylated myosin was more than four times greater (0.126 compared with 0.028) than for non-phosphorylated myosin. The ATPase activity for phosphorylated myosin without added actin (0.052) is increased, reflecting the presence of platelet actin (Fig. 2).

Having found an increase in the actin-activated myosin ATPase activity after phosphorylation, it was interesting to see if dephosphorylation would decrease the enzymatic activity. Myosin was first phosphorylated with $\gamma\text{-}^{32}\text{P}\text{-ATP}$ to contain 0.8-1.0 mol phosphate per mol 20,000 light chain³. Labelled myosin was incubated with *Escherichia coli* alkaline phosphatase which released 70-80% of the covalently bound ^{32}P in 10 h, whereas a control sample incubated in the absence of alkaline phosphatase retained all incorporated ^{32}P .

Both dephosphorylated myosin and the control sample were chromatographed on a column of Sepharose 4B (for the

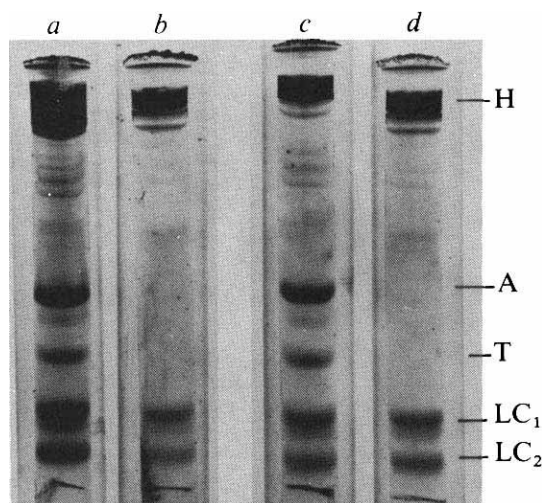


Fig. 2 1% SDS-7.5% polyacrylamide gels of non-phosphorylated (*a*, *b*) and phosphorylated (*c*, *d*) platelet actomyosin (*a*, *c*) and myosin (*b*, *d*) eluted from Sepharose 4B (Fig. 1). Letter above each gel refers to the pooled fraction indicated on the Sepharose 4B elution profile in Fig. 1. H, myosin heavy chain; A, actin; T, tropomyosin; LC₁, 20,000 myosin light chain; LC₂, 15,000 myosin light chain. Dye marker can be seen below LC₂. Migration was from top to bottom. Gel electrophoresis was carried out as described previously¹⁰.

sample treated with alkaline phosphatase, this was necessary to separate the enzyme from myosin). Tubes containing myosin ATPase activity were pooled and assayed for actin activation. Table 2 shows the effect of dephosphorylation on actin activation: a decrease in actin-activated ATPase activity from 0.14 to 0.04 $\mu\text{mol P}_i$ per mg protein per min. The K^+ -EDTA- and Ca^{2+} -activated myosin ATPase activities measured at high ionic strength were not significantly altered. Furthermore, when the two samples were electrophoresed in 1% SDS-7.5%

Table 2 Effect of dephosphorylation on ATPase activity

	ATPase ($\mu\text{mol P}_i$ per mg protein per min)		
	Actin-activated		K^+ -EDTA Ca^{2+}
	-Actin	+Actin	
Phosphorylated myosin	0.037	0.14	0.53 0.55
Dephosphorylated myosin	0.013	0.04	0.40 0.40

Labelling of platelet myosin: conditions for phosphorylation were as in Table 1 except that $\gamma\text{-}^{32}\text{P}$ -ATP was premixed with ATP to give a final concentration of 20 $\mu\text{Ci ml}^{-1}$. Labelled samples were dialysed against 0.5 M KCl, 15 mM Tris-HCl (pH 7.5) and 2.5 mM dithiothreitol before dephosphorylation.

Conditions for dephosphorylation: 2.8 ml labelled myosin (35-55% ammonium sulphate fraction, 9 mg ml^{-1} , 600,000 c.p.m. mg^{-1}) was adjusted to pH 8.0 with Tris base and divided into two equal aliquots. To one, alkaline phosphatase (*E. coli*; BAPF, Worthington) was added in a ratio 1:7 (mg/mg, enzyme/substrate). To the other, an equivalent volume of 65% ammonium sulphate was added. Both samples were incubated at 23 °C for 10-22 h. Incubation was terminated by Sepharose 4B chromatography of the entire sample (1.5 ml) using the same size column and elution buffer detailed in Fig. 1. Concentration of the pooled fraction eluted from the column was 0.3-0.4 mg ml^{-1} . ^{32}P release was measured by precipitating aliquots (50-100 μg) in cold 10% Cl_3CCOOH , 2% sodium pyrophosphate, heating at 96 °C for 20 min, chilling on ice and filtering the precipitate through Millipore HA filters in a Millipore sampling manifold. Filters were washed repeatedly with cold 5% Cl_3CCOOH , 1% sodium pyrophosphate and counted in Aquasol (New England Nuclear) in a Packard Tri-Carb liquid scintillation counter. Identification of 20,000 myosin light chain as the only phosphorylated protein has been described previously³. Conditions for ATPase assay are given in Table 1. Data are from duplicate determinations on three different preparations.

polyacrylamide gels, the patterns were identical. Gel electrophoresis revealed the presence of platelet actin in these preparations, similar to Fig. 2*a* and *c*, and this is reflected in the ATPase activities (Table 2).

Phosphorylation of a light chain from skeletal and cardiac muscle myosin by a kinase isolated from rabbit sarcoplasm has been reported⁶. This kinase differs from that purified from platelets by us in requiring Ca^{2+} for activity⁴. In addition, the platelet myosin light chain kinase does not phosphorylate skeletal or cardiac muscle myosin but does phosphorylate mouse fibroblast⁶ and chicken gizzard myosin⁴.

The identification of actin and myosin in a large number of vertebrate as well as invertebrate non-muscle cells² raises questions as to how the interaction of these proteins is controlled. We have described the effect of phosphorylating the 20,000 light chain of a non-muscle myosin: an increase in the actin-activated myosin ATPase activity. The finding that platelet myosin light chain kinase phosphorylates the 20,000 light chain of fibroblast and smooth muscle myosin suggests that phosphorylation may have a role in controlling actin-myosin interaction in other cells.

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Table 1 Effect of phosphorylation on ATPase activity

		ATPase ($\mu\text{mol P}_i$ per mg protein per min)		
		Actin-activated		K^+ -EDTA Ca^{2+} †
		-Actin	+Actin	
Myosin	Phosphorylated	0.006	0.170	1.00 0.41
	Control	0.002	0.029	0.90 0.38
Actomyosin	Phosphorylated	0.052	0.126	0.54 0.37
	Control	0.007	0.028	0.38 0.38

Conditions for phosphorylation: 1.5 ml 38-52% ammonium sulphate fraction of platelet actomyosin¹ (3.5-6 mg ml^{-1}) was incubated in 0.1 M KCl, 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 4 mM MgCl_2 , 0.2 mM ATP and 2.5 mM dithiothreitol at 23 °C for 30 min (ref. 3) (total volume 1.65 ml). Phosphorylation was terminated by chromatography of the entire sample on Sepharose 4B. Control samples were treated in a like manner with the omission of MgCl_2 and ATP.

* Assay conditions: 10 mM Tris-HCl (pH 7.2), 1 mM ATP, 1.4 mM MgCl_2 and 30 mM KCl. Platelet myosin or actomyosin 0.06-0.10 mg ml^{-1} , rabbit skeletal muscle actin, 0.6-1.0 mg ml^{-1} , 37 °C. Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt⁷ and migrated as a single band in 1% SDS-7.5% polyacrylamide gel electrophoresis and 8 M urea-polyacrylamide gel electrophoresis, pH 8.6. Specific activity excludes added rabbit skeletal actin.

† Assay conditions: 20 mM Tris-HCl (pH 7.2), 2 mM ATP, 2 mM EDTA or 10 mM CaCl_2 and 0.5 M KCl. Platelet myosin or actomyosin, 0.02-0.04 mg ml^{-1} , 37 °C.

Data are from duplicate determination on three different preparations. P_i production was measured by a modification of the method of Martin and Doty⁸. Phosphate production was linear with time during the period used for assay (0-60 min). Protein concentration was determined by the method of Lowry *et al.*⁹ after Cl_3CCOOH precipitation, bovine serum albumin was used as standard.

Oxygen reduction and optimum production of ATP in photosynthesis

THE accepted pathway of CO_2 fixation in plant photosynthesis requires that the photosynthetic light reactions produce ATP and reduced pyridine nucleotide (NADPH) in the molar ratio 3:2 (ref. 1). Early studies of photosynthetic phosphorylation suggested that non-cyclic electron transport could produce only equimolar amounts of ATP and NADPH, and the source of the extra ATP was presumed to be cyclic electron flow². The view that the non-cyclic system is by itself able to produce twice as much ATP as NADPH has been expressed^{3,4}, and removes the need for the *in vivo* operation of a cyclic electron flow which can be demonstrated *in vitro* only in artificial conditions⁵. An inflexible ATP-NADPH ratio of 2:1 for the products of the light reactions would, however, result in a feedback inhibition of electron transport, with ADP concentration as the limiting factor. One way of achieving flexibility in the relative production of ATP and NADPH would be for a low

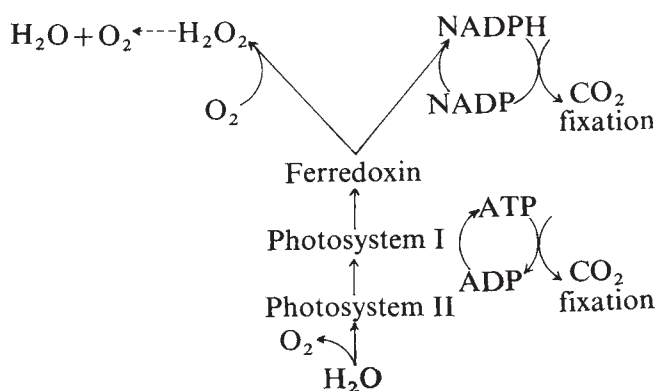


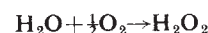
Fig. 1 The combined reduction of oxygen and of NADP by non-cyclic electron transport. The reaction of ferredoxin with oxygen makes possible the synthesis of ATP without the production of NADPH. Stoichiometries are discussed in the text and are not implied in the diagram.

absolute stoichiometry of non-cyclic photophosphorylation (corresponding perhaps to a 1:1 ratio) to be accompanied by an ancillary reaction producing ATP but not NADPH. Such an ancillary reaction would be especially important in situations where ATP might be required for phosphorylations additional to those of the reductive pentose phosphate pathway.

Figure 1 outlines a mechanism by which an optimum balance

of ATP and NADPH could be maintained by non-cyclic electron transport in photosynthesis. Here the ancillary reaction is assumed to be oxygen-reducing electron transport, and the mechanism, therefore, relies on a "pseudocyclic" contribution to overall ATP synthesis, as suggested previously by Heber⁶. The novel feature of this scheme is a competition for reduced ferredoxin by NADP and oxygen. Production of too little ATP would inhibit the dark regeneration of NADP, and ferredoxin would then be oxidised only by oxygen. With oxygen as the effective electron acceptor, ATP but not NADPH would be produced, and a higher concentration of ATP would be restored. Excess ATP would lead to pyridine nucleotide being present predominantly in the oxidised form, and thus to electrons being diverted from oxygen to NADP. In this way the active non-cyclic electron transport chain would continually meet the requirements of CO_2 fixation, and would be able to respond to any changes in the metabolic demands made on it by synthesis of carbohydrate, protein or lipid.

Ferredoxin is an effective mediator of photosynthetic oxygen uptake by isolated chloroplasts⁷, a reaction which accompanies non-cyclic (in this case also termed "pseudocyclic") photophosphorylation⁸. It is also the penultimate component of the photosynthetic electron transport chain, and as such is essential for the reduction of pyridine nucleotide in photosynthesis⁹. The hypothesis represented in Fig. 1 requires ferredoxin to perform both of these functions simultaneously, a situation which is demonstrated experimentally in Fig. 2. In the absence of both NADP and catalase, the rate of photosynthetic oxygen uptake by isolated chloroplasts is a function of ferredoxin concentration (curve *a*), and this oxygen uptake is abolished by addition of catalase (curve *b*). The photosynthetic oxygen-consuming reaction can, therefore, be written as the reversed dismutation of hydrogen peroxide:



This describes non-cyclic transfer of one electron pair from water to oxygen. In the presence of NADP a catalase-insensitive evolution of oxygen should occur as transfer of one electron pair from water to NADP results in the reaction:



In this situation, however, catalase stimulates observed oxygen evolution (curves *c* and *d*), and the degree of this stimulation increases with increasing ferredoxin concentration. This stimulatory effect of catalase on net oxygen evolution can be considered to be no more than an inhibitory effect of catalase on a "background" oxygen uptake. If, of each pair of electrons from water, an average of *n* electrons are transferred to oxygen and 2-*n* to NADP, then the overall reaction occurring in the

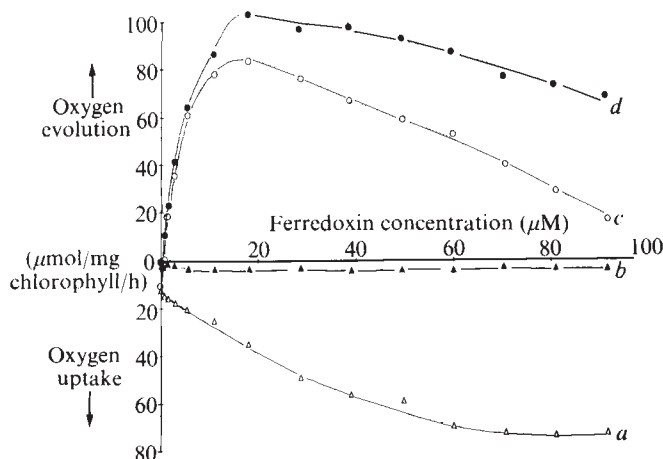
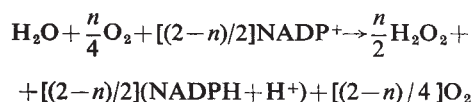


Fig. 2 The effects of catalase on ferredoxin-mediated uptake and overall evolution of oxygen by illuminated spinach chloroplasts. For curves *c* and *d* oxygen evolution was supported by the presence of NADP (2 mM). Curves *b* and *d* represent rates obtained in the presence of 8×10^3 U catalase (Boehringer). For curves *a* and *c* sodium azide (2 mM) was present as an inhibitor of any endogenous catalase.

Oxygen exchange was measured in a Rank oxygen electrode, with illumination by two 300-W slide projectors. Broken, washed chloroplasts had been isolated from spinach by a method described previously¹⁴, and the reaction vessel contained sorbitol (0.1 M), MgCl_2 (5 mM), NaCl (20 mM), EDTA (2 mM), HEPES (pH 7.5, 50 mM), NH_4Cl (5 mM) and chloroplasts (100 μg of chlorophyll) in a final volume of 2 ml. Ferredoxin, isolated from *Spirulina maxima* by the method of Hall *et al.*¹⁵, was added as a 1.4-mM solution. Substantially similar results have been obtained with a number of plant-type ferredoxins (unpublished work). The extent of stimulation of net oxygen evolution by catalase is apparently unaffected both by omission of NH_4Cl and by addition (in the absence of NH_4Cl) of ATP or ADP with phosphate (these results also unpublished).

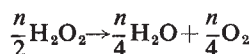
absence of catalase activity (curve *c*) becomes



and the ratio of oxygen molecules evolved to electron pairs transferred is given by

$$(\text{O}_2/2e^-)_{-\text{cat}} = [(2-n)/4] - (n/4) = [(1-n)/2]$$

In the presence of catalase, however, the compensatory reaction



also occurs (curve *d*), and so the corresponding ratio is merely

$$(\text{O}_2/2e^-)_{+\text{cat}} = [(2-n)/4]$$

For any absolute rate of electron transport which is unaffected by catalase, the ratio *r* of the observed rates of net oxygen evolution in the presence ($v_{+\text{cat}}$) and absence ($v_{-\text{cat}}$) of catalase will be given by

$$r = v_{+\text{cat}}/v_{-\text{cat}} = (\text{O}_2/2e^-)_{+\text{cat}}/(\text{O}_2/2e^-)_{-\text{cat}} = (2-n)/[2(1-n)]$$

and thus *n* ($0 \leq n \leq 2$) can be calculated from the relationship

$$n = [1/(1-2r)] + 1$$

In Fig. 3a this measure of the contribution of the oxygen-reducing reaction to total electron flux has been calculated

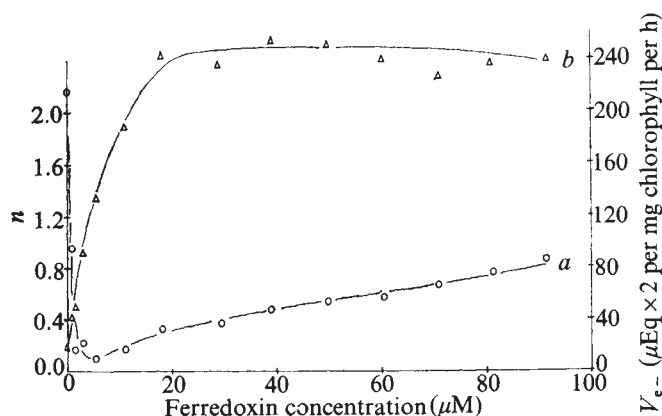


Fig. 3 *a*, *n* is the average number of electrons which go to oxygen, per pair transferred through the photosynthetic chain. *b*, v_{e-} is the rate of electron transport in $\mu\text{Eq} \times 2$ per mg chlorophyll per h, units comparable with similar units involving μatoms oxygen exchanged or μmol NADP reduced. Values for each variable (*n* and v_{e-}) were calculated as described in the text from the data in Fig. 2, with individual values corresponding to observed rates of oxygen evolution at each ferredoxin concentration.

from the data in Fig. 2, and is plotted against the same scale of ferredoxin concentration. As $v_{-\text{cat}}$ is related to the absolute rate of electron transport (v_{e-}) as follows

$$v_{-\text{cat}}/v_{e-} = (1-n)/2$$

the absolute rate of electron flux can also be calculated. The dependence of electron transport *per se* on ferredoxin concentration is shown in Fig. 3b. As in the case of net oxygen evolution (Fig. 2), a saturated rate of electron transport is achieved at a ferredoxin concentration of 15–20 μM . Unlike net oxygen

evolution, however, the rate of electron transport does not significantly decrease with higher ferredoxin concentrations. The decline in the observed rate of oxygen evolution with high ferredoxin concentrations (Fig. 2) presumably reflects an increasing displacement of NADP by oxygen as the terminal electron acceptor (Fig. 3a). At these saturating ferredoxin concentrations of $>15 \mu\text{M}$ $n \approx 0.3$, and so not less than 15% of total electron transport is supported by reduction of oxygen.

These results question Arnon's statement⁸ that in photosynthesis reduction of oxygen cannot occur simultaneously with reduction of NADP. Using mass spectrometry Egneus *et al.*¹⁰ have identified an uptake of $^{18}\text{O}_2$ by CO_2 -fixing chloroplasts in a reaction apparently unrelated to glycolate synthesis. This provides independent support for a competition of oxygen with NADP for electrons from the photosynthetic chain. Whitehouse *et al.*¹¹ have suggested that a significant rate of reduction of oxygen occurs in the presence of added FMN even where the artificial electron acceptor ferricyanide is used instead of NADP and ferredoxin.

The importance to photosynthesis of electron flow to oxygen has also been suggested by Heber, whose detailed measurements of the quantum requirements of various photoreductions support a contribution of oxygen-reducing electron transport to overall ATP synthesis⁶, and point to a flexibility in the stoichiometry of photosynthetic phosphorylation and NADP reduction¹². The work of Patterson and Myers¹³ with *Anacystis nidulans* indicates that an *in vivo* photosynthetic production of hydrogen peroxide may indeed occur.

I thank the SRC for a research studentship. The ferredoxin was a gift from Dr K. K. Rao.

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Agonism and antagonism of γ -aminobutyric acid

EVIDENCE that (+)bicuculline (BIC) is a competitive antagonist of the central inhibitory transmitter γ -aminobutyric acid (GABA) is based¹ mainly on apparent similarities between the molecular structures of BIC, GABA and agonists of GABA. Until recently, there has not been any explanation of the differing potencies of the agonists, or why BIC is an antagonist instead.

In 1970 it was noted¹ that the N and lactone C–C=O in BIC could be isosteric with the N and carboxylate COO of an extended (that is fully *trans*) GABA molecule (zwitterion at physiological pH) and its equivalent (O–C=N) in the agonist muscimol. Alternatively², a partially folded GABA molecule could have its N and COO congruent with the N and lactone

O=C=O of BIC. The discovery of conformationally restricted GABA agonists^{3,4}, notably 4-aminotetrol³, however, gave some support to the extended charge separation—measurements³ on Drieding models gave 5.2–5.8 Å. General agreement was also obtained from EHT MO predictions of minimum-energy conformations for isolated molecules of GABA⁵, muscimol⁵ and other agonists^{6,7}, and for protonated BIC (HBIC—as administered)⁸: a value of 5.8 ± 0.2 Å was obtained⁷ if interchange distances were related to the onium $\rightarrow$ one carboxylate O in the GABA zwitterion and the onium $\rightarrow$

suggests that there is more to its “recognition” of a drug molecule than a single charge separation.

The feature of structure not included in any of the discussions, but now available from CNDO/2 calculations (refs. 9, 22 and unpublished work), is the distribution of charge within the molecules. (Such details were used in the conformation against potency correlation¹² above.) Comparisons of the three-dimensional patterns of charge show features that may distinguish between agonists and antagonists. The charge distribution in the lactone ring in HBIC is of particular interest (Fig. 1a),

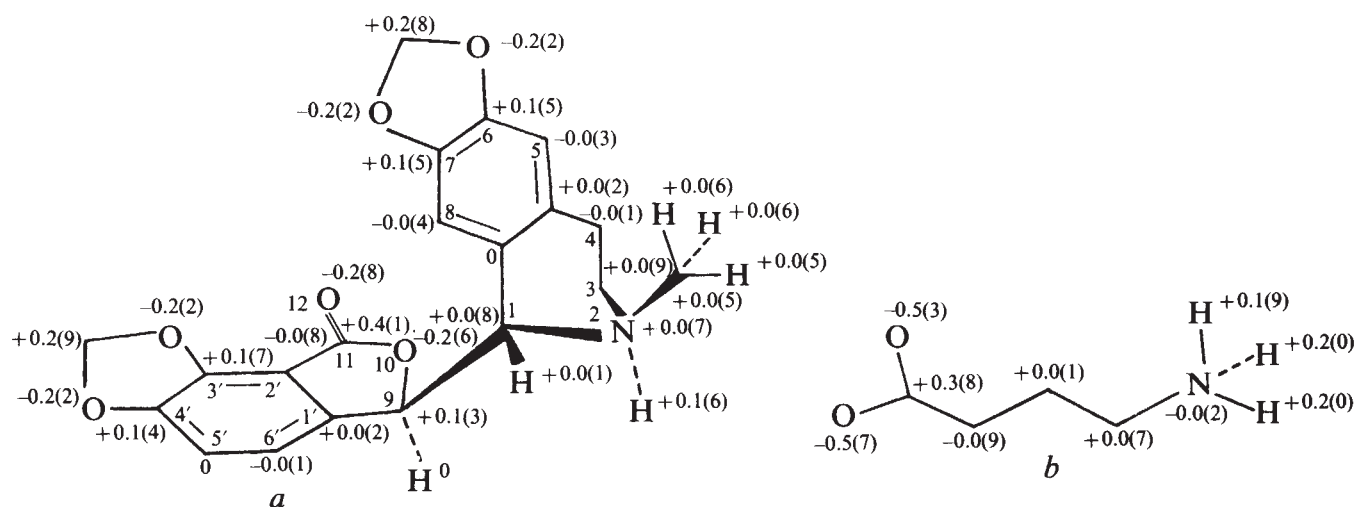


Fig. 1 Skeletal outlines and associated charges (a.u.) for: *a*, HBIC in approximate solution-conformation ($T \sim -90^\circ$); *b*, GABA zwitterion (charges not significantly altered when molecule is partially folded as described in text). Torsion angle $T(N2.C1-C9.O10)$, positive when $N2.C1$ is rotated clockwise to eclipse $C9.O10$, as viewed along $C1 \rightarrow C9$. (H atoms not shown have a charge of 0.0(5) or less.)

carbonyl O in BIC. Although the EHT method is inadequate with strongly polar molecules⁸, and the more folded conformations that would be expected in flexible molecules are predicted by CNDO/2 (ref. 9) and PCIL^{10,11} MO calculations, general agreement with EHT results is fortuitously re-established when allowance is made in the CNDO/2 calculations for an aqueous medium⁹.

A preliminary analysis of the role of agonist flexibility has now revealed¹² a correlation between relative potency and interaction probability calculated from (potential energy) conformation population distributions corrected for the charge-separation tolerance of the receptor.

Concerning BIC as a competitive antagonist, the crystal structure determination^{13,14} of (+)BIC showed^{15,16} that the model originally portrayed¹ was the wrong diastereomer—adlumidine (1S,9S)¹⁷ instead of 1S,9R (ref. 17) (Fig. 1a). Also the methyl substituent at the N is pseudoaxial to the pyridine ring, not pseudoequatorial. Theoretical calculations show, however, that in HBIC^{6,13,18}, and in the potent N-MeBIC^{13,18}, there is appreciable torsional freedom about $C1-C9$ from about -60° to -120° , and within this angular range it is again possible to obtain the isosteric match with GABA which was originally reported¹ for the wrong BIC configuration. Congruence with a partially folded GABA² is not easily attainable but it could be achieved accurately by δ -aminovaleric acid, a GABA agonist¹⁹. The BIC torsion-angle range also makes available a flexibility of charge separation comparable with that of some GABA agonists. BIC should therefore be “recognised” by the GABA receptor as an agonist²⁰, and its action as a competitive antagonist, by which it blocks the permeability change in the postsynaptic membrane, may therefore be due to some further feature of its structure. The report²¹ that (–)BIC is inactive is relevant. Although such selectivity is common, this evidence that the GABA receptor is chirally specific

showing an appreciable difference between the alternatives described earlier, $C2'.C11.O12$ and $O10.C11.O12$; the second bears a closer resemblance to the COO of an amino acid zwitterion. More significant, however, is the smaller charge on the above negatively charged atoms in the lactone ring compared with those in the GABA COO group (Fig. 1b). This, and the differences in the spread of positive charge, are the first intimations of a possible mechanism of competitive antagonism: a charge pattern sufficiently similar to that of an agonist to be “recognised” and attracted to a receptor, but with charges too weak and/or diffuse, or too shielded by the remainder of the molecule, to enable the receptor to bind the drug and then undergo the conformational change believed to be associated with the final stages in the action of a transmitter. The charges on the remainder of the BIC molecule may have a role, perhaps contributing to binding around the receptor site, preventing fast removal of the antagonist molecule and thus blocking the receptor.

Further analysis of the causes of the chiral selectivity of BIC and the mechanism of its antagonism will be aided by results of physiological tests on the other related phthalideisoquinoline alkaloids.

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Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures

ABOUT 99% of all carbon is the ^{12}C isotope while 1% is ^{13}C . The precise ratio of the isotopes will vary depending on the material analysed. In plants, fractionation of carbon is brought

reported for temperate and tropical pastures (ref. 3 and M.M.L., J.H.T., and R.J.J., unpublished). There were also large differences between the ratios for milk and hair produced from C_3 and C_4 pastures, although the mean difference was reduced from -14.0% with pasture to -9.8% for the tissues. This narrowing of the ratio may be due to differences in discrimination against ^{13}C during transformations by the rumen microorganisms or within the tissues of the animal. Alternatively, the animals grazing C_4 pastures may have selectively grazed a small amount of C_3 weeds which would increase the carbon ratio relative to that of the feed sample. Similarly, selection of C_4 weeds in C_3 pastures would reduce the ratio below that expected. Values for milk and hair were identical at three of the four locations, indicating that differences in the carbon ratios of the feeds are reflected in both long term and short term animal products. The cause of the difference in ratios for milk and hair at Swan's Lagoon is unknown. Since the carbon ratio of milk and hair are similar, it is likely that the ratios for other animal tissues, including muscle and fat, will show similar differences between feeds.

These results show that the carbon ratios of animals reflect the carbon ratio of the feed being eaten, and that these ratios can be used as a naturally occurring marker of carbon. This finding could have important implication for animal physiology, forensic science and studies of animal evolution. It should be possible to calculate the proportion of carbon in milk coming from feed and body reserves or to determine the

Table 1 $^{13}\text{C}/^{12}\text{C}$ ratios of pasture and of milk and hair of grazing cattle

Pasture location	Pasture species	Photosynthetic pathway	No. of cows	Time since calving (d)	$\delta^{13}\text{C}$ parts per ml Pasture	$\delta^{13}\text{C}$ parts per ml Milk	$\delta^{13}\text{C}$ parts per ml Hair
Swan's Lagoon, Qld 20°10'S, 147°15'E	<i>Heteropogon contortus</i>	C_4	3	45	-14.0	-15.5	-12.1
Wollongbar, NSW 28°50'S, 153°25'E	<i>Pennisetum clandestinum</i>	C_4	3	107	-12.4	-15.0	-15.1
Murray Bridge, SA 35°07'S, 139°16'E	* <i>Lolium perenne</i>	C_3	1	120	-25.4	-22.5	-22.3
Werribee, Vic. 37°54'S, 144°39'E	* <i>Lolium perenne</i>	C_3	1	210	-28.9	-26.0	-26.2

*Also *Trifolium repens* and other temperate species.

about primarily by carbon dioxide assimilation in photosynthesis and is due to preferential utilisation of ^{12}C and exclusion of ^{13}C . Curiously enough, it has been found recently^{1,2} that higher plants which fix carbon dioxide by way of the Calvin C_3 cycle pathway differ in $^{13}\text{C}/^{12}\text{C}$ ratios from plants which fix carbon dioxide through the C_4 -dicarboxylic acid pathway. Temperate pasture species fix carbon by way of the Calvin pathway and have $^{13}\text{C}/^{12}\text{C}$ ratios of approximately -28% (ref. 3 and M.M.L., J.H.T., and R. J. Jones, unpublished), whereas tropical pasture grasses fix carbon through the C_4 -dicarboxylic acid pathway⁴ and have $^{13}\text{C}/^{12}\text{C}$ ratios of approximately -12% (ref. 2 and M.M.L., J.H.T., and R.J.J., unpublished). $^{13}\text{C}/^{12}\text{C}$ ratios are expressed relative to a carbonate standard⁵.

Smith and Epstein⁶ suggested that isotope ratios of marine animal tissues reflect the ratio in their presumed diet, but there seem to have been no direct comparisons of the isotope ratios of higher animal tissue with those of the food eaten. Our aim, therefore, was to compare the carbon ratios of tissues from animals grazing pasture with contrasting $^{13}\text{C}/^{12}\text{C}$ ratios. The tissues sampled were hair and milk, representing the products of long and short term absorption of nutrients respectively, whereas the pastures grazed were composed of either temperate C_3 species or tropical C_4 grasses, each grazed at two locations (Table 1). Pasture was the only source of carbon except at Wollongbar where 2-3% of the predominantly C_4 diet consisted of bran/oaten chaff (C_3). The isotope measurements of pasture, hair and milk samples were made with a ratio mass spectrometer⁶. Differences between single replicates are usually less than 1%.

The carbon ratios of the pasture samples (Table 1) reflected their botanical composition with values close to those previously

rate of exchange of body protein and fat. This would be achieved by feeding a completely C_4 diet to animals that had previously been given only C_3 feeds so that all their tissues had carbon ratios characteristic of C_3 species. In temperate zones C_4 diet could be based upon either the forage or grain of *Zea mays* or *Sorghum bicolor*. Alternatively, animals on a C_4 diet could be changed to one comprised of C_3 feeds. In forensic science this observation could prove valuable in determining the diet and hence the origin of animals or animal products. It should also be possible to determine the diets of ancient animals by measuring the carbon isotope ratios of their remains.

We thank Ms J. Clark, Dr J. C. Radcliffe and Messrs R. G. Holroyd and A. J. E. Royal for providing the samples of pasture, milk and hair.

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reviews

Makings of a great physiologist

THIS book* is largely directed to a critical account of the development of the ideas and of the experimental research of the great French physiologist, Claude Bernard, during the period 1842–48. From 1841–44 Bernard was *preparateur* to Magendie at the Collège de France but he was already slowly evolving into the great creative investigator that he later became, with ideas and discoveries that ultimately overshadowed those of his teacher. In his discussion of the research Bernard carried out between 1842 and 1848 Professor Holmes has focused on studies of digestion, nutrition and related problems. Although the subject of the study is Claude Bernard much of the book is necessarily concerned with the activities of others, particularly with the adventures in animal chemistry of the two great organic chemists Liebig and Dumas, with their respective supporters.

In his thesis for the Doctorate of Medicine published in 1843 under the title *Du suc gastrique et de son rôle dans la nutrition*, Bernard described the results of investigations which revealed that when sucrose is injected into the veins of a dog it is excreted in the urine largely or completely unchanged; but sucrose given preliminary treatment with digestive juices before its intravenous injection is not so excreted. This was a very important discovery for a young man but afterwards, until 1848, Bernard published few scientific articles.

For a study of Bernard's early scientific development Professor Holmes has been able to consult much manuscript material from the Collège de France, including Bernard's early laboratory notebooks, classified and catalogued between 1962 and 1965 by Professor M. D. Grmek. As Professor Holmes writes in the introduction to his book, "Bernard's notebooks show that the papers he published in this period [1843–48] were only superficial markers of the trail that he was following. . . . His reasoning was often incisive, but occasionally vague, just as his experimental procedures were in some respects unusually rigorous but in

"For more than two years, at the beginning of my career, I wasted my time pursuing theories and chimeras. It was not until after a long period of deception that I ended by reflecting and thinking that . . . my will could not change the laws of nature".

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other respects below the best standards of his time". Professor Holmes's book throws important light on the years of preparation which until now have been very little documented. We can now see for ourselves the evidence for what I suppose most of us guessed at all along, that Bernard must have gone through periods of doubt and made false starts, had prejudices and frustrations, and that in fact he did not spring fully armed into the scientific arena but went through the painful processes of initiation and struggle to which the experimental scientist is naturally subject.

Although Bernard was skilled in experimental surgery his knowledge of chemical methods was much less developed, and his dependence upon the chemical expertise of his colleague C.-L. Barreswil was here of great importance. Barreswil had developed a method for the detection and estimation of glucose by a reduction of an alkaline solution of copper salts which, in many modified forms, had a long life.

In the first chapter of the book, entitled "Chemists, Physiologists, and the Problems of Nutrition", Professor Holmes summarises the conflicting views which prevailed about nutrition in the early years of last century, and some of the controversies in which

Dumas and Liebig figured prominently. In 1841 Magendie presented, on behalf of a commission which had been appointed to adjudicate claims about the alleged special nutritive value of gelatin, a report on 10 years of study. The French Academy were anxious to have an authoritative statement on a topic which was extremely controversial. During the previous century, and subsequently, gelatin had been regarded by some as the basic nutrient substance from which the tissues of the body are formed. The conclusion of the commission, that gelatin in itself is not adequate nourishment, evoked a storm of controversy. But the activities of the commission had an important effect on Claude Bernard since, by carrying out a number of extended feeding experiments for Magendie at the Collège de France, Bernard was initiated into the experimental investigation of animal nutrition. In that way the lectures and writings of Dumas, Liebig and others came to bear on the developing mind of the young Bernard. And the controversies that ensued induced Bernard to ally himself with those who were critical of the uninspired application of chemical methods to the solution of biological problems, although he himself made much use of chemistry in the service of physiology.

**Claude Bernard and Animal Chemistry: The Emergence of a Scientist*. By Frederic Lawrence Holmes. Pp. xx+541. (A Commonwealth Fund Book) (Harvard University Press: Cambridge, Massachusetts, 1974.) n.p.

In 1848 Bernard began the series of investigations on the nature of the processes to which substances absorbed from the gastro-intestinal tract of animals were subjected during their passage through the body. The results of these investigations were summarised in Bernard's thesis for the Doctorate of Science, which was published in 1853 under the title *Nouvelle fonction du foie*. It described his perhaps most famous discovery: the glycogenic function of the liver. From 1853 onwards many publications by Bernard document his scientific development.

Bernard's *Introduction à l'étude de la médecine expérimentale*, published in 1867 as the result of a period of illness which kept him from his lecture room and research bench, gives what can be perhaps an idealised treatment of his subject and of the views that he had assumed by the time he had become an internationally distinguished investigator. Professor Holmes quotes from Bernard's writings later in life the statement that "For more than two years, at the beginning of my career, I wasted my time pursuing theories and chimeras. It is a remark-

able fact, that one imagines that the truth is in himself, and I insisted on repeating experiments which insisted on responding to me always in the same way, contrary to my views. It was not until after a long deception that I ended by reflecting and thinking that . . . my will could not change the laws of nature".

As Professor Holmes states, Bernard's strength was tied to some serious limitations, and his avenue towards greatness was at first a narrow one. There was no guarantee that his will, his insights and his technical skill would lead him to the heights. During the 1840s he was not somebody consciously advancing towards his position of the 1850s and 1860s, but one whose fate was uncertain. "The prospect of returning to Villefranche as a village doctor was as real for Claude Bernard as the prospect of ascent to the Académie des Sciences, the Académie Française and the adulation of his peers".

All who are interested in the way scientists develop will find fascinating material in this study of Claude Bernard during his early years.

F. G. Young

Scientific knowledge and sociological theory

Scientific Knowledge and Sociological Theory. By Barry Barnes. Pp.x+192. (Routledge and Kegan Paul: London, December 1974.) £3.95 cloth: £1.95 paper.

In spite of its Germanic title and subject matter, *Scientific Knowledge and Sociological Theory* is a brief, elegantly written, and even occasionally witty discussion of the social and cultural factors affecting the development of scientific knowledge. It represents an ambitious attempt to apply the perspectives of Mannheim's sociology of knowledge to science.

Barnes rejects conventional distinctions between false beliefs, which require causal explanation, and "naturally reasonable inductions", which do not. He also rejects the definition of science as rational, rather than true, knowledge, on the grounds that there are no adequate criteria for comparing the rationality of different statements: simplicity is ambiguous, and conventional; falsifiability is impossible to establish, and rarely used in practice; and *ad hocery* is merely a derogatory term for secondary elaboration or rational modification. For Barnes, all knowledge is necessarily theoretical, for there is no direct language of observation, and theoretical acceptability is culturally conditioned. Therefore, all knowledge is equally susceptible to sociological explanation.

Normality, defined by the actors themselves, is substituted for truth or ration-

ality as the yardstick for comparing different beliefs; abnormality, rather than falsehood or irrationality, requires causal explanation. "Where actors or groups hold idiosyncratic beliefs as judged against some background of normality, some special cause or condition must be identified which distinguishes that actor or group from that background" (p. 42). Barnes, therefore, focuses upon the maintenance of normality and the development of innovation within scientific sub-disciplines. Normal beliefs are learned through socialisation into the distinctive metaphors and procedures of scientific subcultures, by following exemplary procedures, and by acquiring practical experience. Innovation occurs through differentiation, transfer from one sub-discipline to another, or simply from creativity based upon "a universal human propensity to create and extend metaphors" (p. 87). Such innovations occur partly through the intellectual evolution of conventional paradigms within science itself, and partly through the need to develop new beliefs to fulfil purposes external to science. Thus, the Scientific Revolution resulted partly from the re-emergence of Platonism, and partly from the disintegration of feudalism as a political and social system: "in the general context of post-feudal Europe, teleology had to decline" (p. 117).

The substitution of normality for truth or rationality represents only a formal, if fashionably relativist, advance upon

previous usage. What are the criteria for 'normal'? Is normality to be interpreted in a statistical or cultural sense? Are actors always conscious of the normality or otherwise of their actions? Moreover, rationality can, for 'practical purposes', provide a basis for comparing beliefs in terms of given means-ends relationships. Problems arise with the concept of rationality only when it is impossible to specify ends, that is, at the most general level. But, as Barnes points out, science consists of a number of differentiated subcultures, each with distinctive procedures and ends. The problems of rationality are, therefore, less than Barnes initially suggests.

In general, the sociology of knowledge provides an unsatisfactory basis for explaining scientific knowledge, for the connections between specific beliefs and the social structure remain tantalisingly vague. Barnes argues sensibly that "the sociologist should consider beliefs in terms of their functions in practical activity" (p. 39) and brings out clearly the general factors that might connect science with that activity. But the illustrations of those connections are disappointingly brief and often unconvincing: uniformitarianism as the credo of an emerging industrial order in early nineteenth century Britain and eugenics in Britain before 1914 as a response of the declining ruling class are presented as self evidently plausible examples. At the very least, some attempt at a comparative analysis of alternative reactions is required. Moreover, Barnes shows considerable sympathy for materialist explanations of scientific development (for example, pp. 149-50), but does not discuss the connection between the material bases for the production of science and the content of scientific beliefs in any detail.

The approach to sociology adopted by Barnes is explicitly 'phenomenological', although his use of the approach is valuably flexible. This involves "an appreciation of actors' normal practice as it is, and of its inadequacies as they themselves define them" (p. 43). In practice, however, the author disregards the self imposed limitations this perspective involves, and his analysis of innovation acknowledges the problematic validity of actors' own explanations. This tension, if not inconsistency, stems from the author's recognition that actors' accounts cannot in themselves provide a basis for sociological generalisation and explanation.

In spite of these unresolved problems *Scientific Knowledge and Sociological Theory* represents a valuable and interesting contribution to sociology. At the very least it provides sociologists with an unusual gritty insight into the reality of science. But I doubt whether the book will persuade scientists that sociology has much to offer them in their own 'practical concerns'. Roderick Martin

Finite Groups and Quantum Theory. By D. B. Chesnut. Pp. xiii+254. (Wiley Interscience: New York and London, December 1974.) £7.25.

THIS book satisfies nobody. It is nicely written and clear, but as a physicist I could not possibly recommend it to my students as there are no physical applications. It is indeed remarkable that a book on finite group theory advertised as being suitable for physicists contains no mention of crystal structure. This is presumably because the author is a chemist. But when I asked my colleagues in chemistry for their opinion I was told that the single chapter on the application of group theory to chemical bonding was far too slim and that it could not compete with several other books in the field.

Norman Dombey

Pest Control and its Ecology. (The Institute of Biology's Studies in Biology, No. 50.) By Helmut F. van Emden. Pp. 59. (Edward Arnold: London, January 1975.) £1.90 boards; £0.95 paper.

THIS 59-page book with its list of 17 references is intended to help "teachers and students at school, college or university . . . to keep abreast of recent trends and know where the most significant developments are taking place". It aims to cover primarily the control of insect pests on growing crops and, except for occasional excursions into other hemispheres, it devotes most attention to British insects.

The author deals realistically with the modern vogue for the uncritical condemnation of pesticides, in particular providing yet another disparaging perspective of Rachel Carson's *Silent Spring* which started so many hares running and seemingly also set innumerable hounds into perpetual motion in hot pursuit.

In this book, unfortunately, the possibilities for classical biological control, in the sense of introducing natural enemies of pests of exotic origin, are blurred and diluted by being confused with the mass production of agents for use in flooding techniques. Doubtless, this is because the author lives in a country where there is generally considered to be relatively little potential for the introduction of exotic agents. It is a pity that the more generally applied type of biological control is sold short by mention of such remote or largely misleading concerns as "imported parasitic insects may occasionally carry a crop pathogen externally and thus bring a new problem into the crops" and that its use means that "control is slow, it is not exterminant, unless 'misused', it is often unpredictable, it is difficult and

expensive to develop and apply, and it requires expert supervision" (pp. 19–20).

Those comments do a serious disservice to a flourishing branch of entomology which is pollution-free, involves finite energy input and may produce valuable results lasting long into the future.

In general, however, this book is up to date, eminently readable and conveys much useful information.

D. F. Waterhouse

Books brief

Protein-Calorie Malnutrition. (The Nutrition Foundation: A Monograph Series.) Edited by Robert E. Olson. Pp. xxiv+467. (Academic: New York and London, February 1975.) \$29.50; £14.15.

ALTHOUGH this book contains many papers of a high scientific standard it is difficult to be totally sympathetic to its aims or its achievements. The majority of nutritionists now agree that protein-calorie malnutrition (PCM) can be prevented by eating more food. That being so, symposia on the biochemical and physiological aspects of the disease—which is essentially what this book is—are open to a charge of irrelevance.

Those who accept that metabolic research is still required will find, no doubt, that this book is a useful guide to such projects. They may be irritated by the inclusion of a few papers with virtually no bearing on PCM, and by the fact that a publishing delay of two years has made the contributions somewhat out of date. They will, however, find it a useful text to consult, if it is at times somewhat tedious to read.

It is a pity, though, that the editor did not substitute some practically useful papers for the interminable discussion sections. Then it might have been the important landmark that the Nutrition Foundation claim it to be.

John Rivers

Topics in Carbon-13 NMR Spectroscopy, vol. 1. Edited by George C. Levy. Pp. x+292. (Wiley-Interscience: New York and London, December 1974.) £9.45.

THE remarkable technical advances made over the past five years have allowed access to NMR spectra of dilute spin systems to the extent that ^{13}C spectra are now commonly observed on a routine basis. The considerable advantages of the ^{13}C NMR technique

have engendered much interdisciplinary interest. *Topics in Carbon 13 NMR Spectroscopy* is a useful supplement to existing monographs on the subject. It contains a number of articles by well known authors, which review aspects of the ^{13}C NMR technique. The articles vary from a detailed description of theoretical work, by Professor Ditchfield, which presents some astonishingly accurate calculations of ^{13}C chemical shifts (and which I found most interesting), through articles on relaxation studies (Lyerla and Levy), substituent effects (Maciel), polymer studies (Schaefer), ^{13}C at high-fields (Anet), to dynamic studies (Stothers). The reviewers present well balanced appraisals of their subjects up to late 1973, and generally elucidate the shortcomings of the methods as well as their advantages. Regrettably, that is not the case in the chapter on dynamic studies. In such systems, which are non-linear, the Fourier transform of a free induction decay is not necessarily identical to the continuous wave spectrum for 90° pulse angles, although their identities become closer as the pulse angle decreases; this caveat should have been covered more explicitly than by the inclusion of one reference.

Overall, the volume has much of interest to offer to spectroscopists and to chemists and biochemists generally. I look forward to the continuation of what promises to be a stimulating and informative series.

J. M. Briggs

Flow: Its Measurement and Control in Science and Industry. Vol. 1, part 3: *Flow Measurement and Control.* Edited by W. E. Vannah and H. Wayland. Pp. xliii+1049–1493. (Instrument Society of America: Pittsburgh; Wiley: Chichester, November 1974.) £19.60.

IN order to bring together experts in many branches of fluid flow and to review the most recent advances in the subject, an international symposium was convened in Pittsburgh in the Spring of 1971. Some three years later the Proceedings of that symposium have been issued. All possible facets of flow measurement and control have been considered and the editors have managed to classify the papers into four rather vague themes entitled "Flow characteristics", "Flow measuring devices", "Flow measurement" and "Biological fluid flows". This excellent and ambitious book, comprising as it does a comprehensive range of almost 200 theoretical and experimental contributions, is very well produced and in spite of the high total cost will prove to be the ideal reference source for workers in the entire field of fluid flow.

P. A. Davies

obituary

Ernst David Bergmann, professor of organic chemistry at the Hebrew University and one of Israel's leading scientists, died on April 6 at the age of 71.

Born the son of a Rabbi in Germany, he obtained his PhD at Berlin in 1933, and arrived in London during that year. There he met Chaim Weizmann, with whom he was to form a close working relationship, lasting 18 years. He left for Palestine in 1934 to head the Daniel Sieff Institute (later the Weizmann Institute), which he had helped to plan. Before accepting the professorship of organic chemistry in Jerusalem (where he resigned from the

post of vice-president only last March) in 1953, he had been Scientific Director of the Sieff (Weizmann) from 1934–51. From 1953–66 he was chairman of the Atomic Energy Commission and director of defence research in the Ministry of Defence, and currently had been director of the Harry S. Truman Research Institute at the Hebrew University and Honorary President of the Ben-Gurion University of the Negev. His early studies on the stereochemistry of the Walden inversion and on polycyclic hydrocarbons, and his later work on the toxicity of organic fluorine compounds and on the role of steroids in insect growth, had gained him international fame. His research

had been mainly in the fields of petroleum and photochemistry; on receiving the Solomon Dubnick Prize in 1973, he had predicted the oil crisis that would beset the world, and proposed the establishment of an international agency for oil—along the lines of the International Atomic Energy Agency, in which the Arab states were participants. In recent years he had also been involved in developing contacts between the scientific and technological communities of Israel and West Germany. Among many prizes recognising his lifelong contributions to science, Professor Bergmann had received the Israel Prize for Natural Science and the Rothschild Prize.

announcements

Appointments

The Trustees of the Nuffield Foundation have appointed **John Maddox** as Director of the Foundation. **Robin Matthews** has been appointed a Trustee.

J. R. Parratt, reader in physiology and pharmacology, has been appointed to a personal professorship at the University of Strathclyde.

Birkbeck College, London has appointed **Judith Greene** as professor of psychology.

International meetings

October 2, **Current nutritional developments**, Manchester, UK (Mr J. Crowther, The Metal Box Co. Ltd, Monmouth House, Monmouth Road, Cheadle Hulme, Cheadle, Cheshire SK8 7BJ, UK).

October 6–10, **Nuclear techniques in animal production and health**, Vienna (International Atomic Energy Agency, Kartner Ring 11, PO Box 590, A-1011, Vienna, Austria).

October 6–10, **Remote sensing of environment**, Ann Arbor (The University of Michigan, Extension Service, Conference Department, Ann Arbor, Michigan, 48104).

Person to Person

London accommodation. American pharmacologist and wife, without children or pets, seek small furnished flat (1 or 2 bedrooms) in central London for October and November, 1975, while working on sabbatical leave at University College (Dr J. P. Green, 33 Avenue de Breteuil, Paris 75007, France; tel. no. 734–37–36).

Nuts. Scientist requires small samples of edible nuts without fleshy layer for photographing. Botanical description or description by use. Entry permits will be forwarded (Dr Arnold Krochmal, 13 Veterans Drive, Asheville, North Carolina 28805).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 13–15, **Electronic and aerospace systems**, Washington DC (The Institute of Electrical and Electronics Engineers Technical Activities Board, 345 East 47th Street, New York, New York 10017).

October 13–17, **Gas cooled reactors**, Jalich, German Federal Republic (IAEA/NEA International Symposium on Gas Cooled Reactors with Emphasis on Advanced Systems, c/o International Atomic Energy Agency, PO Box 590, Kartner Ring 11, A-1011, Vienna, Austria).

October 14–16, **CAMAC in computer applications**, Brussels (Dr H. Meyer, Commission of the European Communities, CRC-CBNM, Steenweg naar Retie, B-2440 GEEL, Belgium).

October 16–18, **Platinum**, Denver (G. A. Desborough, Convenor US Geological Survey, Building 25, Denver Federal Centre, Denver, Colorado 80225).

October 20–24, **Safeguarding of nuclear materials**, Vienna, Austria (International Atomic Energy Agency, PO Box 590, Kartner Ring 11, A-1011, Vienna).

October 20–24, **Clean air and pollution control**, Brighton (National Society for Clean Air, 136 North Street, Brighton BN1 1RG, UK).

October 22, **Molecular beam-surface interactions**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 80X, UK).